Case Report

Case-Based Analysis of Pre-analytical and Analytical Non-Conformities in Urinary Protein Testing

Sajjad Bakhtiari[1,2*], Majid Sirati-Sabet[2], Anisha Mathew[3], Vivek Pant[4]

[1]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Clinical Biochemistry, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Department of Biochemistry, Sri Aurobindo Institute of Medical sciences indore, Madhya Pradesh
[4]Department of Clinical Chemistry, Samyak Diagnostic Laboratory, Kathmandu, Nepal

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Corresponding Author:
Sajjad Bakhtiari
Shahid Beheshti University of Medical Sciences
Tehran, Iran
E-mail: sajjadbakhtiari76@yahoo.com

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Abstract

Accurate quantification of urinary protein is fundamental for the diagnosis, monitoring, and management of kidney disease. Despite technological advances, both pre-analytical and analytical non-conformities in laboratory testing remain significant sources of misinterpretation that can adversely affect clinical decision-making. This report analyzes two such cases to highlight common but critical pitfalls in proteinuria assessment. We present two illustrative cases: the first involves a pediatric patient, where a pre-analytical non-conformity led to a significant overestimation of proteinuria severity. The second case describes an elderly diabetic patient where an analytical non-conformity resulted in a profound underestimation of albuminuria. In both instances, discrepancies between semi-quantitative and quantitative results were the critical clues that prompted investigation. These cases underscore that urinary protein results, while quantitative, are not infallible. Vigilant attention to pre-analytical procedures, strict adherence to analytical limits, and the integration of semi-quantitative results as a plausibility check are essential to prevent diagnostic non-conformities. Effective communication between clinicians and laboratory professionals is paramount to ensure that laboratory results accurately inform, rather than misdirect, clinical decision-making.

Introduction

Proteinuria is a key laboratory finding that reflects a wide spectrum of renal and systemic diseases. Accurate measurement of urinary protein is essential for diagnosis, monitoring, and prognosis in kidney disease. However, despite standardized definitions and improved assay technologies, measurement reliability still depends critically on both pre-analytical and analytical factors [1].

Although terminology and classification schemes may vary across clinical and laboratory settings, the core framework generally includes the anatomical source of protein loss, temporal pattern, quantity of protein excretion, and predominant protein type. Recognizing these categories is essential for accurate diagnosis, interpretation of laboratory results, and appropriate clinical management, as each pattern reflects distinct underlying mechanisms and disease implications (Table 1) [2-4].

Among these, microalbuminuria, defined as urinary albumin excretion between 30 and 300 mg/day or its equivalent in spot urine samples, represents an early and sensitive marker of glomerular injury, particularly in diabetic kidney disease and other conditions associated with increased glomerular permeability. Detecting microalbuminuria allows for timely diagnosis and intervention before the onset of overt proteinuria and irreversible renal damage [5].

Laboratory methods for proteinuria assessment encompass a broad range of qualitative, semi-quantitative, and quantitative techniques. These include dipstick analysis, precipitation assays, and more precise turbidimetric, colorimetric, or immunoturbidimetric methods. While each approach offers advantages in terms of sensitivity, specificity, expediency, and cost-effectiveness, they also exhibit limitations and susceptibility to analytical and pre-analytical interferences. Awareness of these factors is essential for accurate clinical interpretation [6].

Despite continuous advancements in assay technologies, both pre-analytical and analytical sources of non-conformities remain significant challenges in accurately evaluating proteinuria. Pre-analytical issues, such as incomplete or improperly timed urine collections, sample mislabeling and contamination, variability in patient adherence to collection protocols, and improper storage or transport conditions, can result in misleading or inconsistent measurements [6].

Analytical challenges can be equally impactful. A critical issue is failure to recognize assay linearity; protein concentrations exceeding the reportable range without proper dilution result in significant underestimation of proteinuria [7]. At the other end, the detection limit of certain methods, such as the sulfosalicylic acid (SSA) precipitation test, may fail to identify mild microalbuminuria, potentially resulting in false-negative findings during early stages of kidney disease [8]. Additionally, common interfering factors such as highly alkaline urine, radiographic contrast agents, elevated concentrations of certain drugs, and substances can adversely affect the accuracy of turbidimetric and colorimetric assays [9]. Moreover, poor harmonization between different analytical methods can yield discordant results, complicating the longitudinal monitoring of a patient's proteinuria [10].

To address these challenges, strict adherence to method harmonization, quality control, and correlation with clinical findings is essential to ensure reliable and clinically meaningful urine protein measurements.

In this report, we present two illustrative cases that highlight critical laboratory challenges in the evaluation of proteinuria. The first case involves a pediatric patient whose proteinuria appeared more severe due to inadvertent use of a 24-hour urine sample instead of a random sample, emphasizing the impact of pre-analytical handling non-conformities. The second case describes an elderly diabetic patient in whom failure to dilute a urine sample exceeding the microalbumin assay's linear range resulted in significant underestimation of albuminuria, illustrating an analytical oversight. These cases illustrate how subtle pre-analytical and analytical non-conformities can distort proteinuria results and demonstrate the value of integrating qualitative and quantitative methods for error detection. We also provide a brief literature review of urine protein measurement methodologies to contextualize these cases within current best practices.

Table 1: Overview of proteinuria categories and their characteristics.

Classification	Categories	Representative Causes and Notes
Source	Pre-renal (Overflow)	Multiple myeloma (light chains), hemolysis (hemoglobinuria), rhabdomyolysis (myoglobinuria)
	Renal – Glomerular	Nephrotic syndrome, glomerulonephritis
	Renal – Tubular	Tubulointerstitial nephritis, acute tubular injury
	Post-renal	Urinary tract infections, prostatic secretions
Temporal Pattern	Transient (Functional)	Exercise, fever, dehydration, congestive heart failure, cold exposure
	Orthostatic (Postural)	Adults (benign)
	Constant	Chronic kidney disease
Quantity	Microalbuminuria	30–300 mg/day albumin; early diabetic nephropathy
	Overt Proteinuria	>300 mg/day total protein
	Nephrotic-range Proteinuria	>3500 mg/day total protein
Composition	Selective Glomerular	Predominantly albumin (e.g., minimal change disease)
	Non-selective Glomerular	Albumin and larger proteins (advanced glomerulopathies)
	Low-Molecular-Weight Proteins	Tubular dysfunction (β2-microglobulin, light chains)

Classification integrates information from KDIGO 2012 Clinical Practice Guidelines [2], Tietz Textbook of Clinical Chemistry and Molecular Diagnostics (8th edition) [3], and Brunzel's Fundamentals of Urine and Body Fluid Analysis (5th edition) [4].

Case Description

Case 1

A 13-year-old girl with a history of persistent proteinuria was referred for reevaluation of renal function and urinary protein excretion. The initial assessment in January 2025 showed a markedly increased random urine protein concentration of 348 mg/dL and a protein-to-creatinine ratio (PCR) of 2.07. The urine protein measurement was performed on a Mindray BS-480 biochemistry analyzer (Mindray, Shenzhen, China) using a pyrogallol red colorimetric method (Ziest Chem Diagnostic, Iran, Lot: 10-545). The manufacturer's reportable range for this assay was 4–120 mg/dL. The initial analysis triggered a ">LIN" (above linearity) flag, indicating the concentration exceeded this range. The 348 mg/dL result was obtained after a manual dilution was performed according to the kit manufacturer's instructions, consistent with overt proteinuria.

At follow-up in early March 2025, a 24-hour urine collection demonstrated total protein excretion of 1848 mg/24 h, confirming significant proteinuria. Urinalysis at that time revealed 2+ protein by the SSA precipitation test (Table 2).

Later that month, during routine follow-up, quantitative analysis of what was recorded as a random urine sample produced unexpectedly high protein (997 mg/dL) and creatinine (407 mg/dL) concentrations, values comparable to those of the previous 24-hour specimen. In contrast, the SSA result remained unchanged at 2+, raising concern about the result's plausibility. A repeat, freshly voided random sample collected on the same day revealed markedly lower protein (182 mg/dL) and creatinine (55 mg/dL) concentrations, confirming persistent but less severe proteinuria.

An internal review determined that the specimen labeled as random urine had been inadvertently drawn from the patient's 24-hour collection container rather than from a new voided sample. The mix-up led to a major overestimation of proteinuria severity and unnecessary clinical concern.

This case underscores the potential for pre-analytical non-conformities to profoundly affect the interpretation of proteinuria severity and highlights the importance of integrating clinical assessment, method comparison, and systematic review of laboratory processes in evaluating unexpected results.

The discrepancy was identified during internal verification, and the corrected result was issued before clinical reporting. Therefore, the error did not influence patient management or clinical decision-making.

Review of internal quality-control (IQC) records for total urine protein assays before and after the incident showed all results within acceptable control limits, and external quality-assessment (EQA) participation confirmed satisfactory performance during the same period.

Following the identification of the specimen mix-up, an internal root-cause analysis (RCA) was conducted. The investigation traced the non-conformity to a lapse in specimen segregation during processing of concurrent random and 24-hour urine samples. The event was documented in the laboratory information system (LIS) as a pre-analytical non-conformity. Corrective actions included staff retraining on specimen labeling and verification, implementation of color-coded collection containers for different urine sample types, and mandatory double-checking of sample identifiers before analysis. These measures were incorporated into the laboratory's quality management plan to prevent recurrence.

Table 2: Serial laboratory and urinary findings in a 13-year-old girl with persistent proteinuria.

Parameter	15 Jan 2025 (Random)	1 Mar 2025 (24-hour)	21 Mar 2025 (Mislabeled†)	21 Mar 2025 (Repeat) (Repeated Random Sample)	Reference Interval (Unit as indicated)
Serum Urea	-	23	26	-	10 – 40 mg/dL
Serum Creatinine	-	0.8	0.7	-	0.5 – 1.1 mg/dL
Random Urine Protein	348	319	997	182	< 15 mg/dL
Random Urine Creatinine	168	46	407	55	20 – 275 mg/dL*
Protein/Creatinine Ratio	2.07	6.93	2.44	3.3	< 0.2 mg/mg
24-hr Urine Protein	-	1848	1054	-	< 150 mg/24h
Urine analysis: Protein (SSA)††	-	2+	2+	-	Negative
Urine analysis: RBCs	-	10–15	10–15	-	< 3/HPF
Urine analysis: WBCs	-	3–4	2–3	-	< 5/HPF
Urine analysis: Granular Casts	-	3–4	4–5	-	Rare or absent/LPF

* Urine creatinine varies by hydration and muscle mass; the approximate expected range is shown.

† This sample was inadvertently collected from the 24-hour collection container rather than a fresh void.

†† SSA (sulfosalicylic acid) grading scale: Negative (<6 mg/dL), Trace (6-30 mg/dL), 1+ (30-100 mg/dL), 2+ (100-200 mg/dL), 3+ (200-400 mg/dL), 4+ (>400 mg/dL).

Dash (-) indicates test not performed.

Abbreviations: SSA, sulfosalicylic acid; HPF, high-power field; LPF, low-power field; RBC, red blood cell; WBC, white blood cell.

Case 2

A 76-year-old woman with a long-standing history of diabetes mellitus was referred for laboratory evaluation due to possible renal impairment. Initial testing revealed fasting hyperglycemia (170 mg/dL), elevated serum urea (56 mg/dL), and increased creatinine (2.5 mg/dL), consistent with reduced renal function. Urinalysis demonstrated 2+ protein by the SSA precipitation test and 1+ glucosuria on dipstick.

Quantitative measurement of urinary microalbumin was performed on a Mindray BS-480 biochemistry analyzer (Mindray, Shenzhen, China) using an immunoturbidimetric assay (AUDIT Diagnostics, Iran; Lot No. 242537). According to the manufacturer's specifications, the reportable range for this method is 2–200 mg/L. The analyzer produced an initial microalbumin result of 249 mg/L, accompanied by a ">LIN" (above linearity) flag, indicating that the concentration exceeded the assay's validated upper limit. However, the flagged result was inadvertently reported without dilution or verification. The unexpectedly modest albumin concentration appeared discordant with the SSA finding of 2+ proteinuria, prompting supervisory review. Upon re-examination, the same specimen was reanalyzed after a 1:20 dilution in accordance with the manufacturer's instructions. The corrected result was 2227 mg/L, confirming severe albuminuria and aligning the quantitative result with the SSA test (Table 3).

This case exemplifies a classic analytical non-conformity in urinary protein measurement, where failure to recognize and correct for assay linearity led to a tenfold underestimation of albuminuria severity. It highlights the critical importance of maintaining awareness of reportable ranges, performing appropriate dilutions, and integrating semi-quantitative results as plausibility checks.

The non-conformity was detected and resolved during supervisory review before result validation, and the corrected report was released to the clinician. Consequently, the incident had no impact on patient care or management decisions.

The analytical non-conformity was documented in the laboratory's non-conformity log as an analytical oversight. A focused RCA revealed that the ">LIN" flag was overlooked during routine result verification. Review of IQC and EQA records confirmed no prior issues with assay linearity or dilution procedures. Corrective measures included refresher training for staff on instrument flag interpretation, reinforcement of mandatory flag acknowledgment in the LIS before result release, and revision of the standard operating procedure (SOP) for handling flagged results.

These interventions were implemented to strengthen analytical vigilance and prevent recurrence.

Table 3: Laboratory and urinary findings in a 76-year-old woman with diabetes.

Parameter	Result	Reference Interval (Unit as indicated)
Blood sugar	170	<140 mg/dL
Serum Urea	56	21-45 mg/dL
Serum Creatinine	2.5	0.6-1.3 mg/dL
Serum Uric Acid	7.6	2.5-6.8 mg/dL
Serum Sodium	144	135–145 mmol/L
Serum Potassium	4	3.5–5.0 mmol/L
Urine analysis: Protein (SSA)†	2+	Negative
Urine analysis: RBCs	10-15	< 3/HPF
Urine dipstick: Glucose	1+	Negative
Urine random microalbumin*	249	< 20 mg/L
Urine random microalbumin**	2227	< 20 mg/L

† SSA (sulfosalicylic acid) grading scale: Negative (<6 mg/dL), Trace (6-30 mg/dL), 1+ (30-100 mg/dL), 2+ (100-200 mg/dL), 3+ (200-400 mg/dL), 4+ (>400 mg/dL).

* Initial result without dilution; exceeded assay upper linearity limit of 200 mg/L.

** Result obtained after 1:20 dilution per manufacturer protocol.

Abbreviations: SSA, sulfosalicylic acid; RBC, red blood cell; HPF, high-power field

Discussion

Accurate urine protein measurement critically depends on the type of specimen collected and meticulous specimen handling during the preanalytical phase. The preanalytical phase encompasses urine sampling, storage, transport, and preparation prior to analysis, involving both patient-dependent and laboratory-controlled steps that significantly impact test reliability and clinical interpretation [6].

Among specimen types, 24-hour urine collection has traditionally been regarded as the gold standard for quantifying proteinuria because it integrates protein excretion over an entire day, accounting for physiological fluctuations [11]. However, drawbacks include patient inconvenience, potential incomplete collection, and handling and storage non-conformities, which can introduce significant inaccuracies [6].

Recognizing these challenges, spot urine samples, particularly the PCR or albumin-to-creatinine ratio (ACR) in first-morning voids or random midstream specimens, have gained wide acceptance in clinical practice as practical and reliable alternatives. Several studies show strong correlations between spot urine PCR/ACR and 24-hour urine protein, supporting their use for screening, diagnosis, and monitoring of renal pathology such as nephrotic syndrome and preeclampsia. Spot samples improve patient compliance and reduce collection non-conformities but require normalization against creatinine to account for urine concentration variability. First-morning void samples are generally preferred to minimize diurnal variation and orthostatic effects, enhancing reproducibility [12,13].

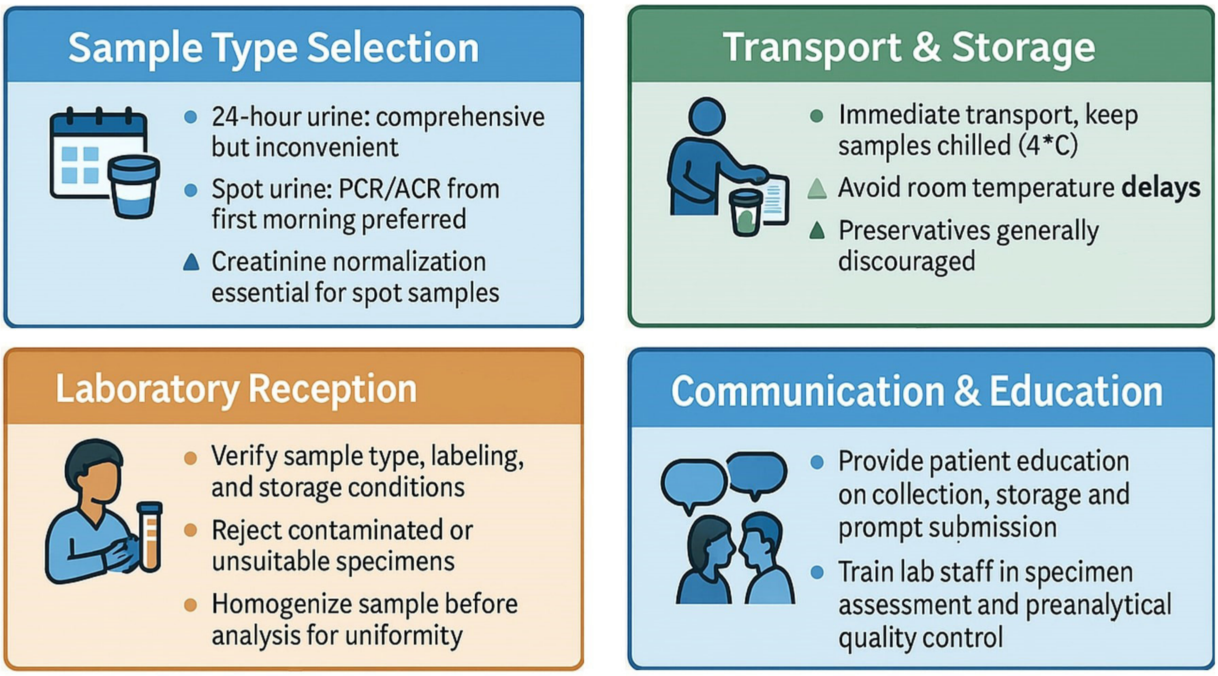
Beyond collection type, specimen handling critically influences the reliability of urine protein measurements. Urine sampling is patient-dependent and susceptible to non-conformities such as contamination with vaginal secretions, menstrual blood, or other extraneous materials, which can cause false elevations or spurious results. Proper midstream clean-catch techniques help minimize contamination [6].

Once collected, urine specimens are biochemically and microbiologically unstable if stored or transported improperly. Proteins can degrade or precipitate, especially when specimens are kept at room temperature for prolonged times. Refrigeration at 4 °C is strongly recommended if immediate processing is unavailable, as it preserves protein integrity and prevents bacterial overgrowth. When transport is required, samples should be kept chilled and delivered to the laboratory promptly to minimize degradation. Studies assessing various preanalytical variables confirm that the stability of urinary proteins significantly declines after extended delays or inappropriate temperature exposure. Furthermore, sample homogenization prior to aliquoting ensures representative protein distribution, critical for accurate measurement [14].

The routine use of chemical preservatives for urine protein is generally discouraged, as they may interfere with specific analytical methods, especially immunoassays. If refrigeration is not feasible, acidification (e.g., with hydrochloric acid) or the addition of preservatives like boric acid may be considered with caution and only when compatible with the intended analytical procedure. Ensuring appropriate storage, labeling, and timely transport remains critical to minimizing pre-analytical variability [15].

The success of accurate urine protein measurement hinges not only on adhering to technical protocols but also on effective communication between laboratory professionals and patients. Proper instruction on urine collection procedures is essential to prevent common pitfalls such as missed voids, sample contamination, or mislabeling. Patients should be clearly educated on the importance of midstream collection, specimen refrigeration, and timely submission. Likewise, laboratory staff must diligently verify specimen type, assess storage conditions, and ensure thorough sample homogenization prior to analysis. Standardized training programs and clear, written instructions are vital to minimizing preanalytical non-conformities and enhancing test reliability across various healthcare settings [6]. A summary of key preanalytical considerations for accurate urine protein measurement is illustrated in Figure 1.

Figure 1: Key preanalytical considerations in urine protein measurement.



This figure summarizes the principal pre-analytical variables that influence the accuracy of urinary protein testing. Critical factors include patient instruction on proper collection technique (midstream, clean-catch, or 24-hour collection), sample labeling and chain of custody, timely transport under refrigeration (4 °C), and avoidance of prolonged storage or chemical preservatives that may interfere with protein stability. Each step can introduce non-conformities if not standardized. Implementing clear protocols and patient–laboratory communication minimizes these errors and ensures valid proteinuria assessment.

Accurate interpretation of proteinuria depends not only on correct specimen collection but also on the selection and performance of the laboratory method used. A wide range of techniques is available for urinary protein measurement, each with distinct analytical characteristics and clinical applications. Understanding these differences is essential, particularly when discrepancies in results occur, as illustrated in our cases.

Initial screening for proteinuria in clinical practice often begins with qualitative or semi-quantitative methods, such as urine dipstick testing or the SSA precipitation test. Dipstick analysis is rapid, inexpensive, and widely available, using a colorimetric reaction to detect primarily albumin via pH-dependent interaction with tetrabromophenol blue. However, it does not detect other protein types such as globulins or Bence-Jones proteins. In addition, its accuracy may be compromised by extreme urine pH, specific gravity, or the presence of interfering substances. While valuable as an initial tool, dipstick testing is best interpreted as part of a broader diagnostic framework, not in isolation [16].

To improve sensitivity to early renal damage, albumin-specific dipsticks or immunochromatographic strips have been developed. These methods target albumin explicitly and demonstrate improved sensitivity for microalbuminuria detection, often with thresholds as low as 2 mg/dL. However, these tests remain semi-quantitative and are also influenced by urine concentration and interfering substances, necessitating cautious interpretation [17].

The SSA test offers a broader protein detection range by precipitating total proteins in urine. This method can detect albumin, globulins, and other high- and low-molecular-weight proteins, and is often used as a semi-quantitative backup to dipstick results. It is particularly helpful when dipstick findings are equivocal or when non-albumin proteins are suspected. However, SSA lacks standardization, and its interpretation is subjective and susceptible to inter-observer variability. It can also produce false-positive results in the presence of contrast media, penicillin, or highly concentrated urine [18]. In our first case, the SSA result provided a critical qualitative flag that was inconsistent with unexpectedly high quantitative measurements, prompting further investigation into sample integrity and specimen handling.

For definitive assessment of proteinuria, quantitative laboratory methods are required. These include turbidimetric and colorimetric assays, which are routinely used in clinical laboratories. Turbidimetric assays, such as those employing benzethonium chloride or trichloroacetic acid, work by precipitating proteins and measuring light scatter. They are cost-effective and suitable for total protein measurement, but are non-specific and vulnerable to interferences. Their linearity range is typically limited to 300–500 mg/dL, and failure to dilute highly concentrated specimens can result in significant underestimation [19].

Colorimetric assays measure urinary protein concentration based on the intensity of color formed when the protein reacts with specific dyes, such as the pyrogallol red–molybdate complex, which offers improved specificity and is widely adapted to automated platforms. These assays are more resistant to chemical interference and have a broader dynamic range, making them well-suited for routine clinical applications. However, like turbidimetric methods, they are primarily suited for total protein measurement and do not distinguish between albumin and other protein types [20].

In patients at risk of glomerular injury, such as those with diabetes or hypertension, measurement of albuminuria using immunochemical methods is essential. Techniques like immunoturbidimetry and immunonephelometry utilize antibodies specific to human albumin and offer high sensitivity, with detection limits as low as 1–2 mg/L. These assays enable early diagnosis of microalbuminuria (30–300 mg/day) and facilitate timely therapeutic intervention before overt nephropathy develops. Nevertheless, their accuracy is highly dependent on proper calibration, reagent integrity, and recognition of their reportable range [21]. As our second case revealed, exceeding this range without proper dilution can lead to gross underestimation, falsely reassuring clinicians and delaying necessary management.

Beyond routine quantitative methods, advanced techniques are employed to provide a more detailed characterization of proteinuria in specific clinical contexts. Urine protein electrophoresis (UPEP) and immunofixation electrophoresis (IFE) allow for the detection and characterization of specific protein fractions, particularly monoclonal immunoglobulin light chains, and are essential tools in the evaluation of suspected paraproteinemias, multiple myeloma, or amyloidosis. These methods, while not quantitative, can distinguish glomerular, tubular, and overflow proteinuria patterns, offering diagnostic clarity when standard assays are inconclusive or discordant [22]. In the research and advanced diagnostic context, mass spectrometry–based proteomic techniques, such as MALDI-TOF or LC-MS/MS, enable the detailed identification and quantification of urinary proteins and peptides. These high-resolution methods support biomarker discovery, disease phenotyping, and insights into renal pathophysiology. However, due to their high cost, technical complexity, and lack of standardization, they are not currently suited for routine clinical use [23].

While advanced techniques provide diagnostic clarity in complex cases, their utility must be weighed against practicality and clinical need. For most patients, a combination of standardized quantitative assays and complementary semi-quantitative methods offers a reliable framework for proteinuria evaluation. As illustrated by our cases, awareness of each method's analytical limitations is crucial for avoiding misinterpretation and ensuring accurate clinical decisions.

Despite advances in assay technologies, inconsistencies between qualitative and quantitative methods and failures to adhere to analytical protocols remain significant sources of non-conformities, as illustrated by our cases. Effective harmonization requires understanding the analytical limitations of each method, implementing standardized procedures, and fostering correlation strategies that integrate multiple data sources for clinical decision-making.

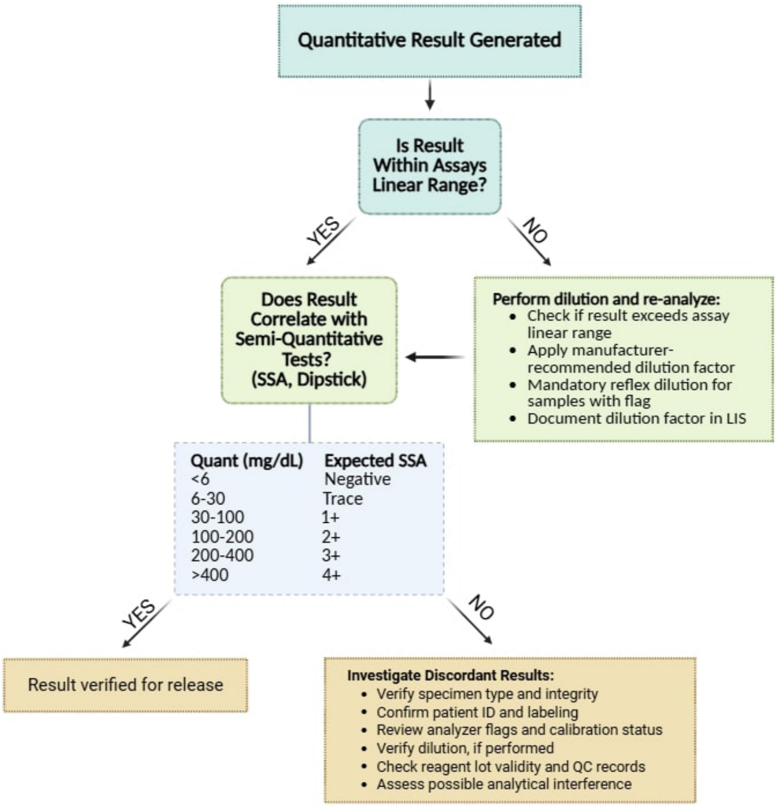
Screening methods such as SSA precipitation and dipstick analysis provide rapid, inexpensive detection of proteinuria, but they are inherently semi-quantitative and subject to variable interpretation. SSA has an approximate detection limit of 5-10 mg/dL, enabling detection of moderate proteinuria but with limited sensitivity for very low-level albumin excretion. For example, a urinary albumin concentration of 35 mg/L (equivalent to 3.5 mg/dL) would typically fall below the SSA detection threshold, resulting in a negative finding despite clinically relevant microalbuminuria. The SSA test provides a semi-quantitative grading that correlates with total urinary protein concentration. According to standard urinalysis references, expected turbidity grades correspond to the following approximate protein concentrations: negative (< 6 mg/dL), trace (6–30 mg/dL), 1+ (30–100 mg/dL), 2+ (100–200 mg/dL), 3+ (200–400 mg/dL), and 4+ (> 400 mg/dL). These ranges can serve as practical plausibility thresholds for verifying the consistency of semi-quantitative and quantitative results [24]. For instance, if a specimen graded as 1+ by SSA corresponds to an expected range of 30–100 mg/dL, a substantially higher or lower quantitative result should prompt verification for potential non-conformities. Incorporating such plausibility thresholds into routine review protocols can help laboratories identify errors early and ensure internal consistency between methods.

In addition to correlating qualitative and quantitative results, maintaining assay linearity and adhering to proper dilution protocols are critical to ensure accuracy in quantitative urine protein measurements. Quantitative assays have defined reportable ranges within which results are considered reliable. Samples with protein concentrations exceeding the upper limit of linearity can yield falsely low results if not appropriately diluted, leading to significant underestimation of proteinuria severity [25]. To address this issue, laboratories should implement multiple safeguards, including automated analyzer flags or LIS alerts for results beyond the linear range, and enforce mandatory dilution procedures as part of standard operating protocols. Comprehensive staff training on manufacturer guidelines and dilution techniques is essential to ensure procedural compliance. Incorporation of automated dilution systems, where available, can further minimize non-conformities and enhance consistency. Furthermore, periodic audits and quality control assessments should be conducted to verify the proper execution of dilution protocols and adherence to linearity criteria. Laboratories should also include confirmatory or reflex testing protocols for borderline or discordant results and educate clinicians about assay limitations to ensure accurate interpretation. Collectively, these measures enhance analytical reliability, reduce the risk of clinically significant misinterpretations, and promote accurate patient diagnosis and management.

A major challenge in urinary protein measurement is achieving consistency across different laboratories, as variability arises from differences in analytical methods, calibrators, and reagent formulations. Turbidimetric, colorimetric, and immunochemical assays often lack full standardization, resulting in significant inter-laboratory bias. This variability can lead to clinical misclassification, particularly when patients transition between care settings or results are compared longitudinally across different facilities [26]. To minimize these discrepancies, laboratories should adopt methods traceable to reference measurement procedures and participate in EQA or proficiency testing programs. Such programs provide benchmarks for identifying systematic deviations and enable corrective actions to align with peer laboratories [27]. The use of commutable reference materials and internationally recognized calibrators is essential to reduce method-dependent variability [28].

A multimodal approach that combines standardized assays, rigorous quality control, and proactive clinician-laboratory communication is essential to ensure accurate diagnosis, timely intervention, and improved patient outcomes. A proposed workflow for ensuring accurate urinary protein results is shown in Figure 2.

Figure 2: Algorithmic workflow for verification of urinary protein results.



Perform dilution and re-analyze:
- Check if result exceeds assay linear range
- Apply manufacturer-recommended dilution factor
- Mandatory reflex dilution for samples with flag
- Document dilution factor in LIS

Quant (mg/dL)	Expected SSA
<6	Negative
6-30	Trace
30-100	1+
100-200	2+
200-400	3+
>400	4+

Investigate Discordant Results:
- Verify specimen type and integrity
- Confirm patient ID and labeling
- Review analyzer flags and calibration status
- Verify dilution, if performed
- Check reagent lot validity and QC records
- Assess possible analytical interference

The workflow outlines key decision steps for validating quantitative urine protein results. It incorporates automated detection of values exceeding the assay's linear range, mandatory reflex dilution and re-analysis with documentation in the laboratory information system (LIS), and plausibility cross-checking against semi-quantitative methods such as the sulfosalicylic acid (SSA) test. Expected SSA ranges serve as reference thresholds for assessing consistency. Discordant findings prompt review of specimen integrity, labeling, and analytical performance before result release.

Lessons Learned and Recommendations for Laboratory Implementation

SOP Modifications

- Color-coded labeling: Use distinct labels for 24-hour vs. random specimens
- Mandatory dilution protocols: Establish automatic dilution for samples with flags
- Concordance verification: Routinely cross-check SSA/ dipstick with quantitative results
- Supervisory review: Require approval before releasing flagged or discordant results

LIS Enhancements

- Automated alerts: Flag multiple specimen types from the same patient within 24 hours
- Result release blocks: Prevent reporting when linearity exceeded without dilution
- Dilution documentation: Mandate entry of dilution factor before result authorization
- Specimen tracking: Implement barcode verification at collection and aliquoting

Staff Education and Competency

- Initial training: Comprehensive modules on specimen handling and flag recognition
- Annual refresher courses: Competency testing on dilution techniques and result verification

Quality Assurance Measures

- Monthly audits: Review all results with linearity flags for dilution compliance
- EQA participation: Include high-concentration samples to challenge upper linearity
- Periodic validation: Verify dilution protocols and concordance criteria quarterly
- Clinician feedback: Establish communication channels for unexpected results

Patient Education

- Written instructions: Provide clear collection guidelines for 24-hour specimens
- Visual aids: Use diagrams showing proper collection techniques
- Verbal reinforcement: Have staff review collection procedures with patients

Conclusion

Accurate urinary protein measurement is essential for reliable assessment of renal function, yet remains vulnerable to both pre-analytical and analytical non-conformities. The two cases presented illustrate how specimen misidentification and failure to recognize assay linearity can profoundly distort clinical interpretation. These findings emphasize the necessity of rigorous specimen verification, adherence to assay performance limits, and continuous quality assurance. Integrating semi-quantitative plausibility checks and strengthening clinician–laboratory communication are key strategies to prevent diagnostic error. Ongoing efforts toward harmonization and staff training are critical to ensure that urinary protein results consistently support, rather than compromise, patient care.

Ethic statement

The study protocol, including the presentation of clinical case data and associated laboratory findings, was reviewed and approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Tehran, Iran). Written informed consent was obtained from the patient and the legal guardian for publication of de-identified clinical information, laboratory data, and tables derived from their results. All identifying details were removed to ensure confidentiality in accordance with institutional and international ethical standards for human subject research.

Data availability

The data supporting the findings of this study are derived from clinical case records and laboratory results of individual patients. To protect patient confidentiality, these data are not publicly available. De-identified excerpts relevant to the cases may be shared by the corresponding author upon reasonable request and with permission from the institutional ethics committee.

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Conflict of interest

The authors declare that there is no conflict of interest concerning this study.

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