Methods for Diagnosing Proteinuria—When to Use Which Test and Why: A Review

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Proteinuria plays a central role in the diagnosis of kidney disease and has a high prognostic value. The test methods used differ considerably regarding their impact on test accuracy, sensitivity, and specificity. Therefore, knowledge of the methodology is crucial for the interpretation of the results. In addition to the distinction between semiquantitative and quantitative tests, there are also relevant differences within the 2 methods. In general, semiquantitative tests are easy to handle but have limitations such as incomplete quantification, a lack of specificity regarding the type of proteinuria, and a high rate of false-positive results that require retesting with a quantitative method for verification. In contrast, quantitative methods, especially immunoassays, have the advantages of high test accuracy and the possibility of targeted detection of specific protein molecules in addition to albumin. However, these methods are more expensive and require access to a laboratory or an electronic point-of-care device. In this Review, the different types of tests for proteinuria and their underlying methodologies and strengths and weaknesses are discussed in detail to allow a rational decision of use and the correct interpretation of the results depending on the clinical context.

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Introduction

As early as Hippocrates (~460-370 BC), it was stated that "when bubbles settle on the surface of the urine, they indicate disease of the kidneys and that the complaint will be protracted." Nearly two millennia later, F. Dekkers of Leiden, The Netherlands (1648-1720), described a method of detecting what is now called proteinuria by adding acid to the urine for the coagulation and precipitation of protein in solution.[1] Currently, various methods are in clinical use to quantify and to determine the nature of proteinuria. Understanding the different methods is the basis for selecting the appropriate test and interpreting the results. The aim of this Review is to explain how the most common methods work on a chemical or immunochemical level. The Review also discusses the importance of semiquantitative and quantitative tests for clinical use and which tests are most suitable for screening, diagnosis, and monitoring of proteinuria, taking into account their limitations.

Semiquantitative Methods to Detect Proteinuria

Nonspecific Colorimetric Dye–Based Semiquantitative Tests

Semiquantitative urine dipstick tests are popular for population screenings and routine clinical check-ups and are often used in ambulatory settings, particularly in low- to middle-income countries, where rapid, low-cost diagnostic methods are essential.[2-5] The numerous commercially available dipstick devices rely on the "protein error of pH indicator dyes" principle. The binding and complex building of protein and the dye leads to a gradual color change, even though the pH of the solution is unchanged.[6-9] The dissociated dye anion reacts with the positively charged side chains/amino groups of the protein, whereby an equilibrium exists between the dissociated dye anion and the dye–protein complex. The gradual color change depends on the concentration and affinity of the protein to the anionic dye. Therefore, tests are more reactive to albumin than other proteins because albumin contains more positively charged amino groups.[8-11] Dipsticks are highly buffered, so changes in pH of the solution should not significantly alter the color of the indicator. Still, the major source of false-positive results are strongly alkaline urine samples (pH >9) that override the buffer system.[10] The causes of alkaline urine samples are found in therapies with phenazopyridine, chloroquine, chlorhexidine, chinidine, or nitrofurantoin, in addition to contamination by detergents or antiseptic agents in the urine sample.[10] False-negative findings occur when the urine is too diluted or if the proteinuria is characterized by proteins other than albumin (Fig 1A).[10,12]

Albumin-Specific Colorimetric Dye–Based Semiquantitative Tests

Besides the multi-dipstick test for protein detection, semiquantitative tests are available for more specific albuminuria detection. Microalbustix test strips (Siemens Healthcare Diagnostics Ltd) employ a combination of a semiquantitative indicator dye–based measurement of albumin (10, 30, 80, and 150 mg/L) using a high-affinity sulfonephthalein dye and a semiquantitative measurement of urine creatinine (Ucr) concentration (10, 50, 100, 200, and 300 mg/dL) based on a peroxidase-like activity of a copper–creatinine complex that catalyzes the reaction of diisopropylbenzene dihydroperoxide and

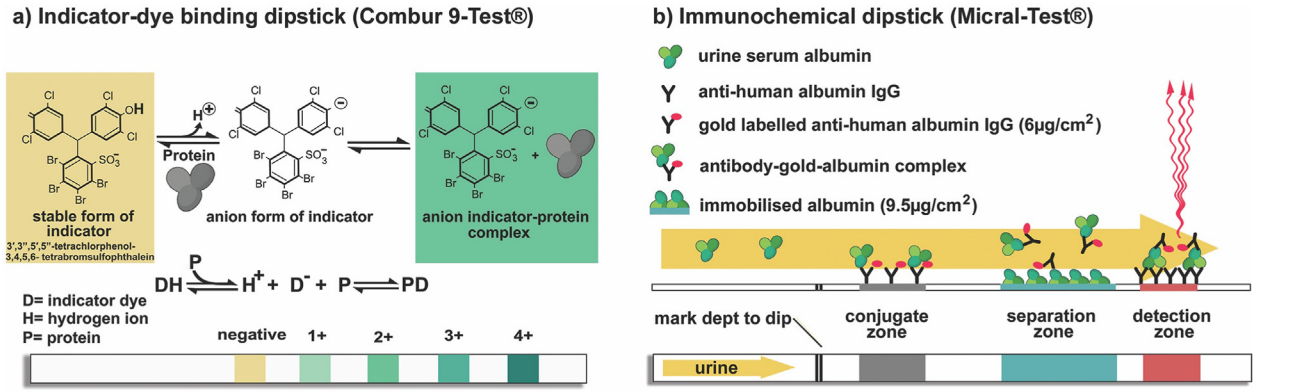


Figure 1. Principles of semiquantitative urine dipstick diagnostics. (A) Indicator dye–based dipstick test. (B) Immunochemical-based dipstick test with gold-labeled anti-human albumin antibody.

3,3',5,5'-tetramethylbenzidine. The colors of the reagent areas of both tests are read visually by comparing them versus a color chart. The urine albumin-creatinine ratio (UACR) is determined according to a table. For example, a ratio of 80 mg/L albumin to 200 mg/dL creatinine corresponds to a UACR of 40 mg/g (4.5 mg/mmol), with the manufacturer's recommendations specifying a cutoff value of ≥3.4 mg/mmol (≥30 mg/g) for abnormal albuminuria (for conversion from mg/g to mg/mmol, see Table 1).[13,14]

Albumin-Specific Chromatographic Immunological Semiquantitative Tests

The albumin-specific semiquantitative dipstick test called the Micral-Test (Roche Diagnostics) is a chromatographic immunological test based on a colloidal gold–labeled monoclonal immunoglobulin G antibody (6 µg/cm^2) that is highly specific to albumin.[10] With the correct amounts of urine absorbed, the soluble antibody–gold–albumin conjugate is transported to the separation zone containing fixed human albumin (9.5 µg/cm^2), where excess gold-labeled anti-human albumin immunoglobulin G is retained. This allows only the conjugate–albumin immunocomplex to reach the detection zone. As the colloidal gold nanoparticles refract light, the detection pad changes color from white to red in proportion to the amount of albumin in the urine.[10,15,16] The Micral-Test detects the lowest levels of albuminuria, with a detection range of 2-10 mg/dL albumin, and is therefore able to detect the earliest signs of albuminuria (Fig 1B).[5,10]

Table 1. UACR and UPCR Threshold Values for CKD Stages A1-A3

CKD Stage	UACR[a]	UPCR[a]
Normal to mildly increased (A1)	<30 mg/g (<3 mg/mmol)	<150 mg/g (<15 mg/mmol)
Moderately increased (A2)	30-300 mg/g (3-30 mg/mmol)	150-500 mg/g (15-50 mg/mmol)
Severely increased (A3)	>300 mg/g (>30 mg/mmol)	>500 mg/g (>50 mg/mmol)

Abbreviations: CKD, chronic kidney disease; UACR, urine albumin-creatinine ratio; UPCR, urine protein-creatinine ratio.
[a]Conversion factor from mg/g to mg/mmol is 0.113; conversion from mg/g to mg/mmol: multiply by 0.113; conversion from mg/mmol to mg/g: divide by 0.113; in clinical use and in international guidelines, the numbers are rounded, eg, 30 mg/g × 0.113 = 3.4 mg/mmol and corresponds to ~3 mg/mmol.

Quantitative Methods to Detect Proteinuria

Quantitative Measurement by 24-Hour Urine Collections and the Meaning of Urine-Creatinine Reference Values

For decades, 24-hour urine collection and the resulting quantification of total proteinuria was considered the reference method for assessing and classifying protein excretion by the kidney.[17] The correct 24-hour urine collection should start with an empty bladder. From that time point onward, all subsequent voidings should be collected over a time period of 24 hours, including a last emptying of the bladder.[18] To minimize protein degradation, the collected urine should be stored in a dark, cool place at a constant temperature, eg, in a refrigerator, until it is handed over to the laboratory. This tedious sampling procedure is susceptible to errors, resulting in imprecise collections and estimates of proteinuria.[19,20] To determine the adequacy of 24-hour urine collection, the Ucr excretion can be assessed because, in a steady state, daily Ucr excretion is relatively constant and in proportion to muscle mass.[21] This is supported by a recently published article showing that, in healthy participants, the Ucr excretion rate (in mg/h) was similar for most of the sampling times, whereas the urine flow rate (in ml/h) and Ucr concentration (in mg/dL) vary over time.[22] Estimates from a retrospective household survey with 1,463 participants aged 20-79 years, which was conducted in Germany in 1986-1988, found mean creatinine totals in a 24-hour urine sample of approximately 11 mmol (0.14-0.18 mmol/kg/24 h) in women and approximately 15 mmol (0.18-0.21 mmol/kg/24 h) in men, slightly decreasing with age and notably higher in obese patients

Table 2. Clinical Examples of the Relationship Between UACR, UPCR, and 24-Hour Urine Albumin and Protein Excretion

Condition	UACR[a]	UPCR[a,b]	Albumin Excretion in 24 h[c]	Protein Excretion in 24 h[c]
Moderate increased albuminuria	300 mg/g (30 mg/mmol)	~500 mg/g (~50 mg/mmol)	~300 mg	~500 mg
Severely increased albuminuria	700 mg/g (70 mg/mmol)	~1,000 mg/g (~100 mg/mmol)	~700 mg	~1,000 mg
Nephrotic-range proteinuria	2,200 mg/g (220 mg/mmol)	~3,500 mg/g (~350 mg/mmol)	~2,200 mg	~3,500 mg

Abbreviations: CKD, chronic kidney disease; UACR, urine albumin-creatinine ratio; UPCR, urine protein-creatinine ratio.
[a]Conversion factor from mg/g to mg/mmol is 0.113; conversion from mg/g to mg/mmol: multiply by 0.113; conversion from mg/mmol to mg/g: divide by 0.113; in clinical use and in international guidelines, the numbers are rounded, eg, 30 mg/g × 0.113 = 3.4 mg/mmol and corresponds to ~3 mg/mmol.
[b]The relationship between UACR and UPCR is an approximation: there is no conversion factor because the test methods are different (UACR vs UPCR) and the amount and composition of proteins vary when measuring total protein.[52,60]
[c]Based on the assumption that the average creatinine excretion rate is approximately 1.0 g/24 h or 10 mmol/24 h, a UACR of 300 mg/g (30 mg/mmol) and a UPCR of 500 mg/g (50 mg/mmol) correspond to approximately 300 mg/24 h and 500 mg/24 h, respectively.

(body mass index ≥30 kg/m^2) of either sex.[21] The difficulty of accurate urine collection was pointed out in a retrospective study of 381 collected 24-hour urine specimens: 51% were reported to be inaccurate with 14% of patients presenting an overcollection and 37% an undercollection based on Ucr excretion reference ranges of 15.0-20.0 mg/kg/24 h (0.13-0.18 mmol/kg/24 h) for women and 18.0-24.0 mg/kg/24 h (0.16-0.21 mmol/kg/24 h) for men.[19]

During the past decade, investigators have pointed out that these "general nephrology reference ranges" should be regarded with caution.[23,24] Forni Ogna et al reported that the reference ranges are likely outdated because they are based on studies and observations from the 1960s and 1970s[25,26] that were conducted in generally leaner and younger populations than found today in Europe.[23] In addition, the reference ranges used as clinical standards do not take into account the individual's age, weight, and ethnicity, which are known factors to have influence on Ucr excretion.[23,25,27,28] Therefore, to improve the accuracy, Forni Ogna et al developed nomograms by means of a derivation population and an independent validation cohort of Europeans with an estimated glomerular filtration rate >60 mL/min/1.73 m^2. The nomograms include tables for age and body mass index to provide more personalized and accurate ranges for Ucr excretion per 24 hours. The authors emphasized the use of the concept in clinical practice and epidemiologic studies.[29] However, to the best of our knowledge, the nomograms developed have not yet been applied in any study.

Quantitative Measurement by Spot Urine Samples

With increasing awareness of the limitations of 24-hour urine collection and on the background that the relatively constant Ucr excretion allows a quantitative assessment of the protein excretion in a spot urine sample independently of the knowledge of urine volume, the urine protein-creatinine ratio (UPCR) and the urine albumin-creatinine ratio (UACR) were introduced[30] and showed good concordance with 24-hour proteinuria measurements (Table 2).[30-33]

To obtain quantitative UPCR or UACR results, measurements of Ucr, total protein, or specific albumin concentration are required. Detection is based on one of 2 methods: a measurement of turbidity that measures the reduction in the intensity of light transmitted through a sample due to scattering and absorption by suspended particles within the sample, or with nephelometry, in which light is refracted at a target molecule and deflected onto a detector that is outside the direct path of the transmitted light. The turbidity or optical density can only be measured if the difference between incident and detected light is sufficiently large. This depends primarily on the concentration of the probe but can also be influenced by the particle size. At low concentrations at which the optical density is not significantly different, the nephelometric approach is superior because the incident light is scattered and refracted by the target molecule even if the concentration is low (Figs 2A and 2B). Turbidimetry has a very high degree of automation, is less expensive, and is resistant to interference, which is why it represents the standard in clinical chemistry, including the measurement of albumin and total protein (with the exception of cerebrospinal fluid with very low albumin concentrations). Because of the disadvantage of limited automation and for cost reasons, nephelometry is not suitable for a 24-7 service, but can cover a wide range of particle size and is highly sensitive and is therefore preferred for samples with low particle concentrations, eg, tubular proteins.

To assess the creatinine concentration, an enzymatic colorimetric reaction is used in which creatinine is transformed to sarcosine and urea by creatininase and creatinase. Adding sarcosine-oxidase results in the formation of glycine, formaldehyde, and hydrogen peroxide (H_2O_2) in an exact ratio to creatinine. Under the catalytic reaction of peroxidase, the quinoneimine dye is formed in stoichiometric proportion to the available H_2O_2. The quinoneimine dye's color intensity is colorimetrically assessed at 546 nm (main wavelength) and is directly proportional to the creatinine concentration. The converted concentration can be used to calculate the UPCR or UACR. Creatinine concentration can also be determined using the colorimetric Jaffe reaction, whereby creatinine reacts with picric acid in

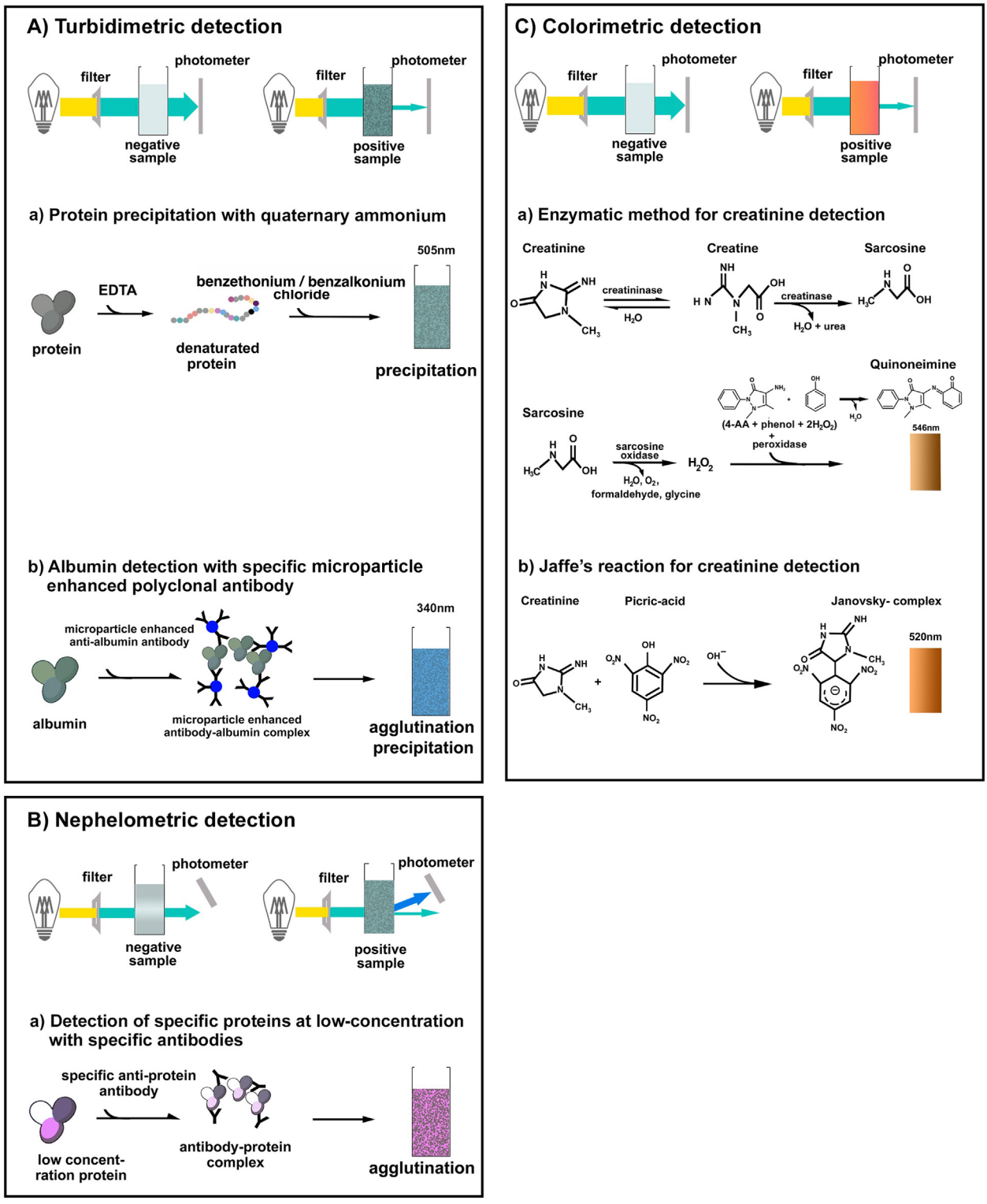


Figure 2. Principles and applications for quantitative photometric urine diagnostics. (A) Turbidimetry is the measurement of turbidity (optical density), ie, reduction in the intensity of light transmitted through a sample. (a) Precipitation with quaternary ammonium for turbidimetric total urine protein quantification. (b) Detection of albumin with specific polyclonal anti-albumin antibody for turbidimetric quantification; to increase the degree of turbidity, the anti-human albumin antibodies are fixed to latex particles. (B) Nephelometry is the measurement of deflected light outside the direct path of transmitted light. (a) Detection of specific proteins at low concentration with specific anti-target protein antibodies for nephelometric quantification, eg, for tubular proteins. (C) Colorimetry is the measurement of color change. (a) Enzymatic reaction for colorimetric creatinine quantification. (b) Jaffe's reaction for colorimetric creatinine quantification. 4-AA, 4-aminoantipyrine; IgG, immunoglobulin G.

Table 3. Overview of Discussed Semiquantitative and Quantitative Methods for Diagnosing Proteinuria and Their Clinical Applications and Limitations

	Semiquantitative			Quantitative				
	Unspecific Protein	Specific Albumin		Total Protein	Albumin	Proteins in Low Concentration	Creatinine Reference	
Chemical or immunochemical principle	Complex building of protein and indicator dye	Specific colloidal gold–labeled anti-albumin Ab	Protein: binding to indicator dye and creatinine (catalytic reaction)	Quaternary ammonium precipitation	Specific anti-albumin Ab	Specific anti-target protein Ab	Enzymatic method	Jaffe's reaction with picric acid
Method	Colorimetry	Colorimetry	Colorimetry	Turbidimetry	Turbidimetry	Nephelometry	Colorimetry	Colorimetry
Ratio to creatinine	No ratio	No ratio	Semiquantitative albumin-creatinine ratio	Total protein-creatinine ratio	Albumin-creatinine ratio	Ratio for specific proteins, e.g. α-1-microglobulin–creatinine ratio	Prerequisite for calculation of UACR, UPCR, and any ratio to creatinine	Prerequisite for calculation of UACR, UPCR, and any ratio to creatinine
Clinical application	High NPV to rule out proteinuria; suitable to rule out proteinuria if no quantitative test available	High NPV to rule out albuminuria; suitable to specifically rule out albuminuria if no quantitative test available	Limited data; not suitable for screening purposes due to low sensitivity	Assessment of proteinuria if no UACR available; if UPCR and UACR performed simultaneously, detection of abnormally high gap between total protein and albumin may indicate paraproteinuria	Gold standard for screening and classification per KDIGO guidelines	Determination of specific indicator proteins at low concentrations, eg, tubular proteins (suitable, eg, for patients with suspected tubulointerstitial damage, eg, drug-induced interstitial nephritis)	Enables concentration-independent quantification of total proteinuria, albuminuria, and other urinary proteins	Enables concentration-independent quantification of total proteinuria, albuminuria, and other urinary proteins
Limitations/ disadvantage	High false positive rate with low PPV; high rate of retesting with quantitative test for confirmation; limited specificity for albumin	Very high false positive rate with very low PPV; very high rate of retesting with quantitative test for confirmation; higher test costs than nonspecific dipstick tests	Limited data; high false negative rate; risk of missing positive cases	Less accurate quantification vs Ab-based tests; no further characterization of proteins; lack of standardization; higher susceptibility to test interference; need for laboratory access	Tubular proteins missed; higher test costs vs dipsticks; need for laboratory access or electronic point of care	Need for laboratory access	No relevant limitations	Interference by substances with alteration of test accuracy

Abbreviations: Ab, antibody; KDIGO, Kidney Disease: Improving Global Outcomes; NPV, negative predictive value; PPV, positive predictive value; UACR; urine albumin-creatinine ratio; UPCR, urine protein-creatinine ratio.

an alkaline solution, resulting in the orange-red Janovsky complex, which is colorimetrically read at 520 nm (main wavelength) and is directly proportional to the creatinine concentration (Fig 2C).[34,35]

This reaction was first described by German biochemist Max Jaffe in 1886.[36] For clinical application in nephrology, the Harvard University scientist Otto Folin developed the creatinine quantification method within the first 2 decades of the 20th century,[34] and the method is, to date, considered the oldest clinical method still in use.[35]

Compared with the enzymatic method, however, the Jaffe method has a higher susceptibility to interfering substances.[37] Possible substances identified for interaction include acetoacetate, acetone, ascorbate, pyruvate, and cephalosporines.[37] Another factor in interactions is diabetes mellitus, especially if poorly controlled with high glucose and hemoglobin $A1_C$ levels or increased levels of β-hydroxybutyrate, which is why an enzymatic method should be used instead in diabetic patients.[37] Furthermore, interactions are observed at low or normal creatinine ranges rather than at high creatinine concentrations, as well as in delayed sampling processes, the latter likely due to the accumulation of pyruvate.[37,38] Finally, it should be emphasized that the enzymatic method can also be affected by interfering substances, albeit to a lesser degree than the Jaffe method, which is why interference characteristics need to be carefully evaluated before the tests are used in the clinic (Table 3).[37,39]

Quantitative Measurement of Proteins in High Concentration

Measurement of total protein was historically performed using acid-induced precipitation followed by assessment of the turbidity of the precipitate. Later, cationic quaternary ammonium detergents were shown to produce turbidity with serum proteins at alkaline pH and to be more stable than acid-induced precipitates.[40] An alkaline solution (ie, EDTA) is added to the urine sample to denature proteins and eliminate interfering magnesium ions, and then benzethonium chloride or benzalkonium chloride is added in the alkaline milieu and turbidity is measured turbidimetrically at a wavelength of 505 nm (main wavelength). The intensity of light absorbed is used to determine the concentration in the sample using a standard calibration curve as a reference (Fig 2Aa; Table 3).

A highly specific polyclonal anti-human albumin antibody targeting various albumin-specific epitopes is used to determine the albumin fraction in the urine sample. When albumin is present, the antigen–antibody complex agglutinates and can be measured turbidimetrically at a wavelength of approximately 340 nm. To increase the degree of turbidity, the anti-human albumin antibodies are fixed to latex particles (Fig 2Ab; Table 3).

Quantitative Measurement of Proteins in Low Concentration

For the detection of proteins in low concentration, eg, α_1-microglobulin, retinol-binding protein, transferrin, immunoglobulin G and α_2-macroglobulin–specific antiprotein antibodies are used for the nephelometric determination (Fig 2Ba; Table 3).

What Test to Use and Why

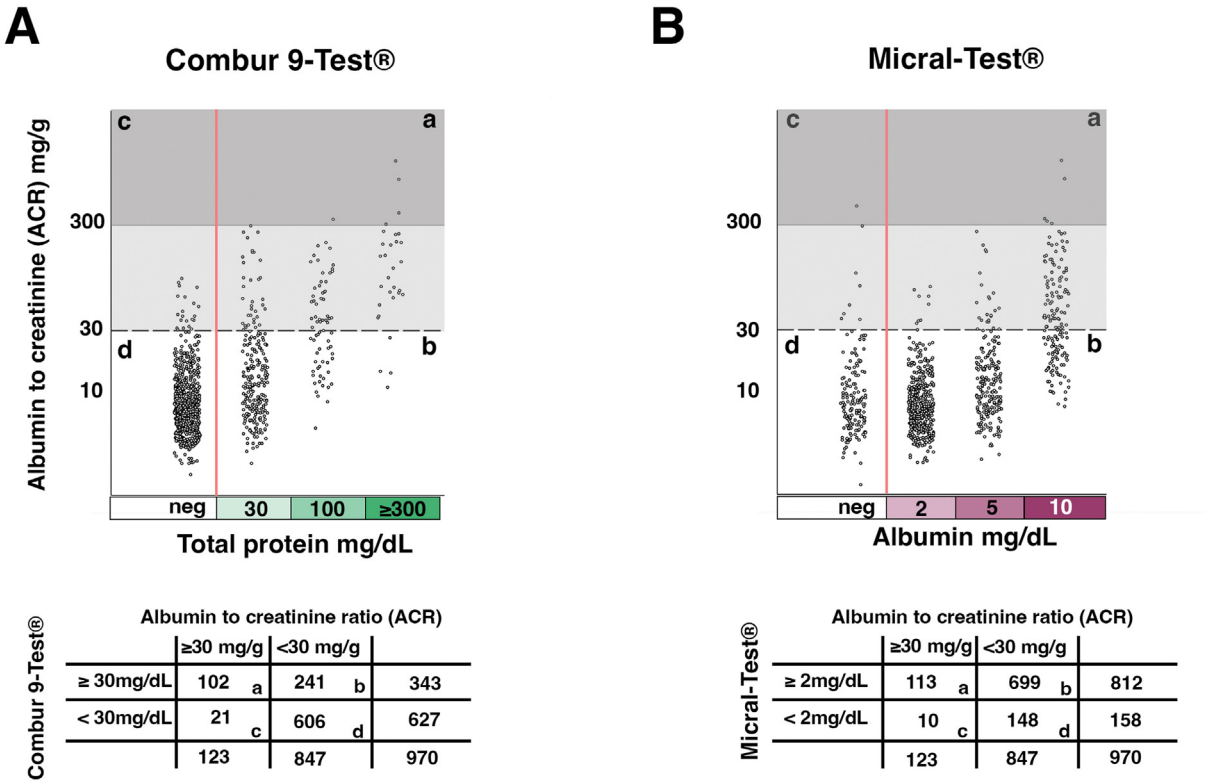
Semiquantitative Tests for Proteinuria

The usefulness of semiquantitative urine dipstick tests has been investigated in numerous studies.[5,41-47] In general, the prevalence of the event or disease within a surveyed population is a critical measure because prevalence rates influence the positive and negative predictive values of a test, especially when continuous measures are dichotomized with cutoff values.[48-50] Often, predictive values are more relevant to the clinician or policymaker than sensitivity and specificity measures alone, which address the accuracy of the screening test relative to the reference test, whereas predictive values indicate the effectiveness of the screening test to discriminate between healthy and affected individuals.[48,49] Therefore, if some authors did not report the prevalence of proteinuria detected by the reference test, these studies are limited, particularly in terms of predictive value.[41,43] Another difficulty in comparing the studies arises from the heterogeneity of the patient cohorts that were used to assess the different semiquantitative urine dipstick tests and reference tests.[5,41-47]

In a study from Croatia, 75 urine samples were used to compare the 12 most commonly used dipstick tests in the country.[43] The reference test was the Combur-10 Test M (Roche Mannheim Germany) with the indicator dye 3′,3″,5′,5″-tetrachlorphenol-3,4,5,6-tetrabromsulfophthalein for the detection of urinary protein.[10,43] The study found a positive agreement between the different dipstick tests for total protein measurements, with strong κ-values ranging from 0.79 to 0.93 and high levels of reproducibility (repeatability was assessed on 20 replicates of each dipstick brand for all tests).[43] When compared versus the quantitative turbidimetric method with benzethonium chloride and a dipstick cutoff level set at ≥1+, sensitivity was generally >80% with a wide range of heterogeneity among the tests (55.9%-91.7%), and specificity was generally low, ranging from 41.5% to 72.2%.[43] The authors concluded that the dipsticks have a suboptimal accuracy for total protein detection.[43]

In a general population from Korea comprising 20,759 dipstick tests, results were compared versus a quantitative UACR test. For the detection of an ACR ≥30 mg/g (≥3.4 mg/mmol), the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of a dipstick ≥ trace were 43.6%, 93.6%, 34.6%, and 95.5%, respectively.[42] A similar finding came from a landmark study in a general population in Australia in which 10,944 patients were surveyed.[45] For dipstick result trace positivity detecting a UACR ≥30 mg/g (≥3.4 mg/mmol), the PPV was 47.2% (95% CI, 43.9%-50.5%) and the NPV was 97.6% (95% CI, 97.2%-97.9%).[45]

We found similar results in a prospective study from a walk-in population in which we surveyed 970 patients

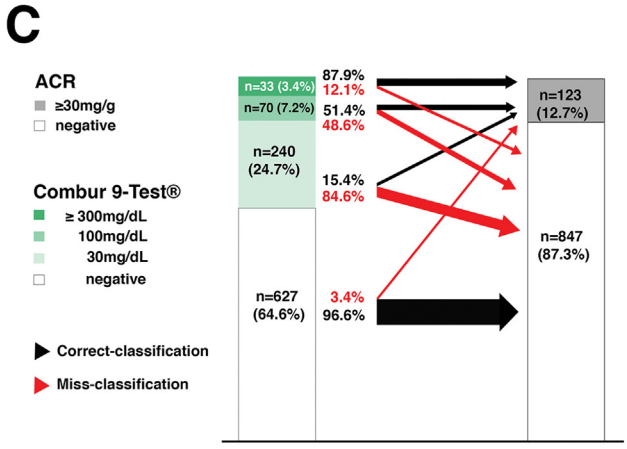


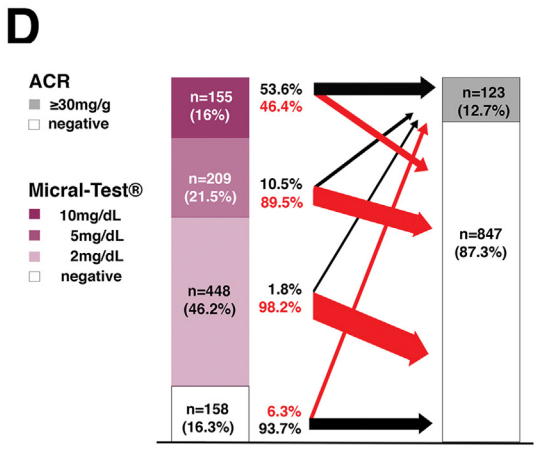
Albumin to creatinine ratio (ACR)

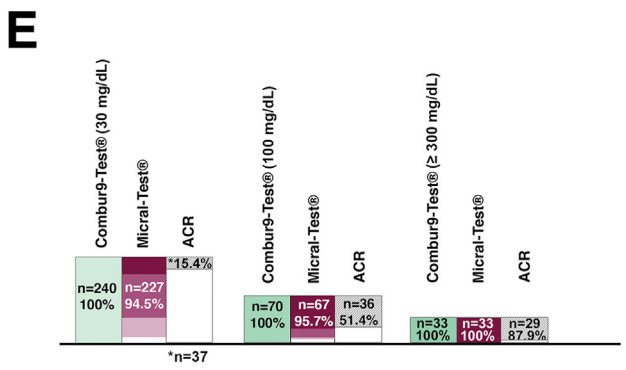
Combur 9-Test®	≥30 mg/g	<30 mg/g	
≥ 30mg/dL	102 a	241 b	343
< 30mg/dL	21 c	606 d	627
	123	847	970

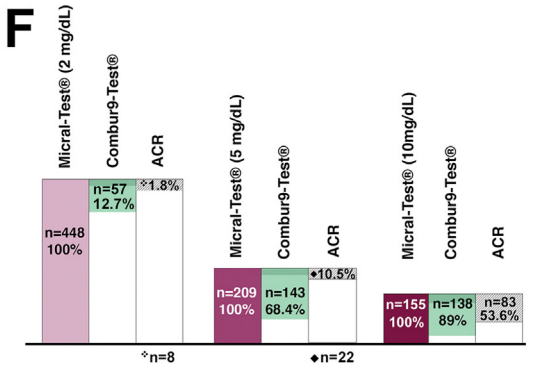
Albumin to creatinine ratio (ACR)

Micral-Test®	≥30 mg/g	<30 mg/g	
≥ 2mg/dL	113 a	699 b	812
< 2mg/dL	10 c	148 d	158
	123	847	970









from semirural sub-Saharan Africa with an estimated albuminuria prevalence of 12.7% (95% CI, 10.6%-14.8%; Fig 3).[5,51] We compared 2 different semiquantitative dipstick tests versus a quantitative UACR test.[5] The colorimetric dipstick Combur-9 Test had a low PPV of 29.7% (n = 102/343; 95% CI, 24.9%-34.9%) at the lowest cutoff level (≥30 mg/dL; ≥1+) but was sufficient for ruling out patients with a negative test result (n = 606/627; NPV, 96.6%; 95% CI, 94.9%-97.9%; Figs 3A and 3C). With the highly albumin-specific immunochromatographic Micral-Test, the PPV for the lowest cutoff value (≥2 mg/dL) was very low at 13.9% (n = 113/812; 95% CI, 11.6%-16.5%). At first sight, the NPV also appeared strong at 93.7% (n = 148/158; 95% CI, 88.7%-96.9%; Figs 3B and 3D). However, despite a similarly high NPV, the proportion of patients in whom albuminuria could be ruled out was significantly lower with the Micral-Test (15.2%; n = 148/970) than with the Combur-9 Test (62.4%; n = 606/970; Figs 3C and 3D). Adjusting the cutoff level of the Micral-Test from 2 mg/dL to 5 mg/dL resulted in an NPV of 97% (95% CI, 95.4%-98.2%) and a comparable proportion of patients (60.6%; n = 588/970) in whom albuminuria could be ruled out (not shown).[5] Based on a positive test result, Fig 3E (positive Combur-9 Test) and Fig 3F (positive Micral-Test) show the relationship between the 2 dipstick tests and between the dipstick tests and the reference test (UACR ≥30 mg/g) at different cutoff values.[5]

Only limited data are available in which Microalbustix urine test strips, which provide a semiquantitative measurement of UACR, are compared to a quantitative UACR test. In a small cohort of patients with type 2 diabetes, 12.5% of whom had microalbuminuria (reference UACR >28 mg/g [2.5 mg/mmol] in men and >39 mg/g [>3.5 mg/mmol] in women), the sensitivity, specificity, PPV, and NPV of the Microalbustix strips were 33.3%, 92%, 40% and 89.6%, respectively.[13] Compared with conventional semiquantitative tests with a high rate of false-positive results, the Microalbustix strips carry the risk of missing a relevant proportion of true positives, as the rate of false-negative results was high (Table 3).

According to guideline recommendations, any positive dipstick test requires a proteinuria workup with a quantitative method.[52] Under these premises, the high false-positive rate of semiquantitative dipstick tests would generate excessive follow-up quantitative testing, as shown in our own study (25% with the Combur-9 Test [≥30 mg/dL], n = 241/970; and 72% with the Micral-Test [≥2 mg/dL], n = 699/970; Figs 3A and 3B).[5] Therefore, even though there is still controversy in regard to the utility of dipstick tests in clinical practice, authors agree that semiquantitative urine dipstick tests are suitable only to rule out proteinuria by a negative test result (Table 3).[5,13,17,42,45,52,53]

UACR or UPCR to Test for Proteinuria

The question arises whether UPCR or UACR is the appropriate quantitative test method (Table 3). Methodologically, it is more difficult to standardize the assessment of total protein than to specifically measure urinary albumin immunochemically to determine a UACR.[17] A recently published study undertaken by the Chronic Kidney Disease Prognosis Consortium including 919,383 adults from 12 research cohorts and 21 clinical cohorts demonstrated that the overall concordance of UPCR and UACR tests was good from same-day sample measurements.[54] However, even though the relationships between urine samples assessed with UACR and UPCR for proteinuria were nearly linear on the log scale for UPCR values >50-500 mg/g (>5.6-56 mg/mmol), no consistent relationship was found for a UPCR <50 mg/g (<5.6 mg/mmol).[54] In the AusDiab study in a general population in Australia with 10,596 urine samples, 2.4% were found to have a positive UPCR result (≥200 mg/g [≥23 mg/mmol]), and, of these, 91% had a positive UACR result (≥30 mg/g [≥3.4 mg/mmol]), whereas, among all samples with a positive UACR result (6.8%), only 32% had a positive UPCR result, illustrating the high sensitivity and specificity of UACR for the detection of albuminuria.[55]

In a primary-care population, 569 urine samples were used to investigate the discrepant finding of a positive UPCR test (≥200 mg/g [≥23 mg/mmol]) and a negative UACR test (<30 mg/g [<3.4 mg/mmol]) in the same specimen.[56] Albuminuria detected by a UACR test in the absence of proteinuria may be expected because of the higher sensitivity of the immunochemical UACR test,

Figure 3 (previous page). Correlation, agreement, and relationship between the colorimetric indicator dye–based urine Combur-9 Test and the immunochromatographic albumin-specific urine Micral-Test with the urine albumin-creatinine ratio (UACR) reference test (≥30 mg/g [≥3.4 mg/mmol]).[52] (A and B) Correlation of the Combur-9 Test and Micral-Test with UACR: (a) dipstick- and UACR-positive, (b) dipstick-positive and UACR-negative, (c) dipstick-negative and UACR-positive, and (d) dipstick- and UACR-negative. The dashed horizontal black line indicates the UACR cutoff value for positive testing at ≥30 mg/g (≥3.4 mg/mmol), the white area indicates albuminuria <30 mg/g (<3.4 mg/mmol), the light gray area indicates albuminuria 30-299 mg/g (3.4-34 mg/mmol), and the dark gray area indicates albuminuria ≥300 mg/g (≥34 mg/mmol). The red lines indicate dipstick-negative results versus dipstick-positive results. Agreement/disagreement of the Combur9-Test (C) and Micral-Test (D) with UACR: black arrow, agreement; red arrow, disagreement. The arrow widths are proportional to the percentage of agreement/disagreement. (E and F) Relationship between the 2 dipstick tests and the reference UACR ≥30 mg/g (≥3.4 mg/mmol) based on a positive Combur-9 Test (E) or a positive Micral-Test (F). Color codes and numbers and percentages are as in C and D. Adapted from Hodel et al.[5]

whereas the opposite (ie, proteinuria without albuminuria) seems more unusual. Intuitively, this would point in the direction of tubular disturbance or any protein leaking into the urine from the urothelial tract or the presence of paraproteinuria causing proteinuria. Therefore, the authors ran the discrepant samples in a subsequent analysis using a highly sensitive sodium dodecyl sulfate agarose gel electrophoresis technique.[56] Although the control samples of patients with moderately increased UACR were clearly positive for albumin with strong bands around 66 kDa, the 27 discrepant probes were negative or only weakly positive for albumin (<30 mg/g [<3.4 mg/mmol]), illustrating the sensitivity of the sodium dodecyl sulfate agarose gel electrophoresis technique for urinary proteins.[56] Although 10 of the discrepant samples had an increased α1-microglobulin–creatinine ratio and 4 of these also had an increased β-trace protein–creatinine ratio, the amount was too small to explain the discrepancy between positive UPCR and negative UACR. The authors could not identify the cause of this discrepancy. The presence of peptides in the urine that react with the total protein assay but were present in concentrations too low to be visible on sodium dodecyl sulfate agarose gel electrophoresis was considered unlikely. In addition, there was no evidence of excess excretion of immunoglobulins, including light chains. The authors concluded that an artificial interference was the most probable cause and suggested that UACR, rather than UPCR, should be the primary test for proteinuria (Table 3).[56]

According to KDIGO (Kidney Disease: Improving Global Outcomes), an initial evaluation for chronic kidney disease can be based on UACR or UPCR.[52] However, total protein measurement is problematic because of the large sample-to-sample variations in the amounts and compositions of proteins, variable concentrations of nonprotein interfering substances, and lack of standardization. Therefore, the UACR is the preferred test to be used for screening, diagnosis, and monitoring of proteinuria.[52,57,58] If UPCR is measured, UACR should also be measured for further specification because albumin is the most important protein for the investigation of glomerular damage. If tubular proteinuria is suspected, a specific marker protein (eg, α_1-microglobulin) should be determined by immunostaining because tubular proteins may be hidden in the normal gap between UPCR and UACR as a result of their low concentrations (Tables 1 and 3). If the gap is above the expected range, paraproteinuria should be considered. However, if paraproteinemia is clinically suspected, it should always be investigated because a "normal" gap never rules it out.

Summary

Tests for proteinuria vary methodologically, with significant implications for sensitivity, specificity, and accuracy. Therefore, knowledge of the methodology is crucial for the interpretation of the results depending on the clinical context. Semiquantitative urine stick tests are very popular because of their low cost, ease of use, and short time requirements. However, clinicians and policymakers should be aware of the limitations, particularly the high false-positive rates that would require retesting by a quantitative method for verification.[5] In contrast, quantitative immunoassays have high test accuracy and do not need verification by a second method, but are more expensive and require access to a laboratory or point-of-care device. However, from a cost and clinical effectiveness perspective, proteinuria screening should not be based on an initial semiquantitative dipstick test, but on a quantitative method, preferably with the determination of a UACR.[42,59] If no quantitative test is available, a dipstick test can be used to rule out proteinuria, but not for screening purposes.

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