

QUANTITATIVE ESTIMATION OF PROTEIN IN URINE BY SULPHOSALICACIC ACID METHOD

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QUANTITATIVE PROTEIN ESTIMATION

- *In a healthy renal and urinary tract system*, the urine contains no protein or only trace amounts.
- *The presence of increased amounts of protein* in the urine can be an important indicator of renal disease. It may be the first sign of a serious problem and may appear before any other clinical symptoms.
- *However*, there are other physiologic conditions (eg, exercise, fever) that can lead to increased protein excretion in urine. Also, there are some renal disorders in which proteinuria is absent.

PROTEINURIA

- *Protein in normal urine* should be less than **150 mg/L**
- *Proteinuria* is defined as urinary protein excretion of **greater** than **150 mg per day**(per one liter)
- **Note!!!**: Dipsticks (is the most common *initial* screening test for proteinuria) can only detect around 150 mg/L of albumin (**The dipstick** will not detect nonalbumin proteins)
- **Note** : /L = /24-hour = /day



Types of proteinuria

Type	Cause
Glomerular proteinuria	Results from a disruption of the glomerular filtration barrier which increased filtration of normal plasma protein and because albumin has the highest concentration in the plasma it is called abuminuria eg. Malignant hypertention
Tubular proteinuria	Defect in the reabsorption eg, Fanconi Syndrom Low molecular weight protein that is found in urine
Overflow proteinuria	Overflow of high plasma concentrations of low molecular weight protein found in urine eg, Multiple myeloma
Secretory proteinuria	Over secretion of certain proteins in the tubules, most notably the over secretion of Tamm-Horsfall proteins eg, in interstitial nephritis

TYPES OF PROTEINURIA

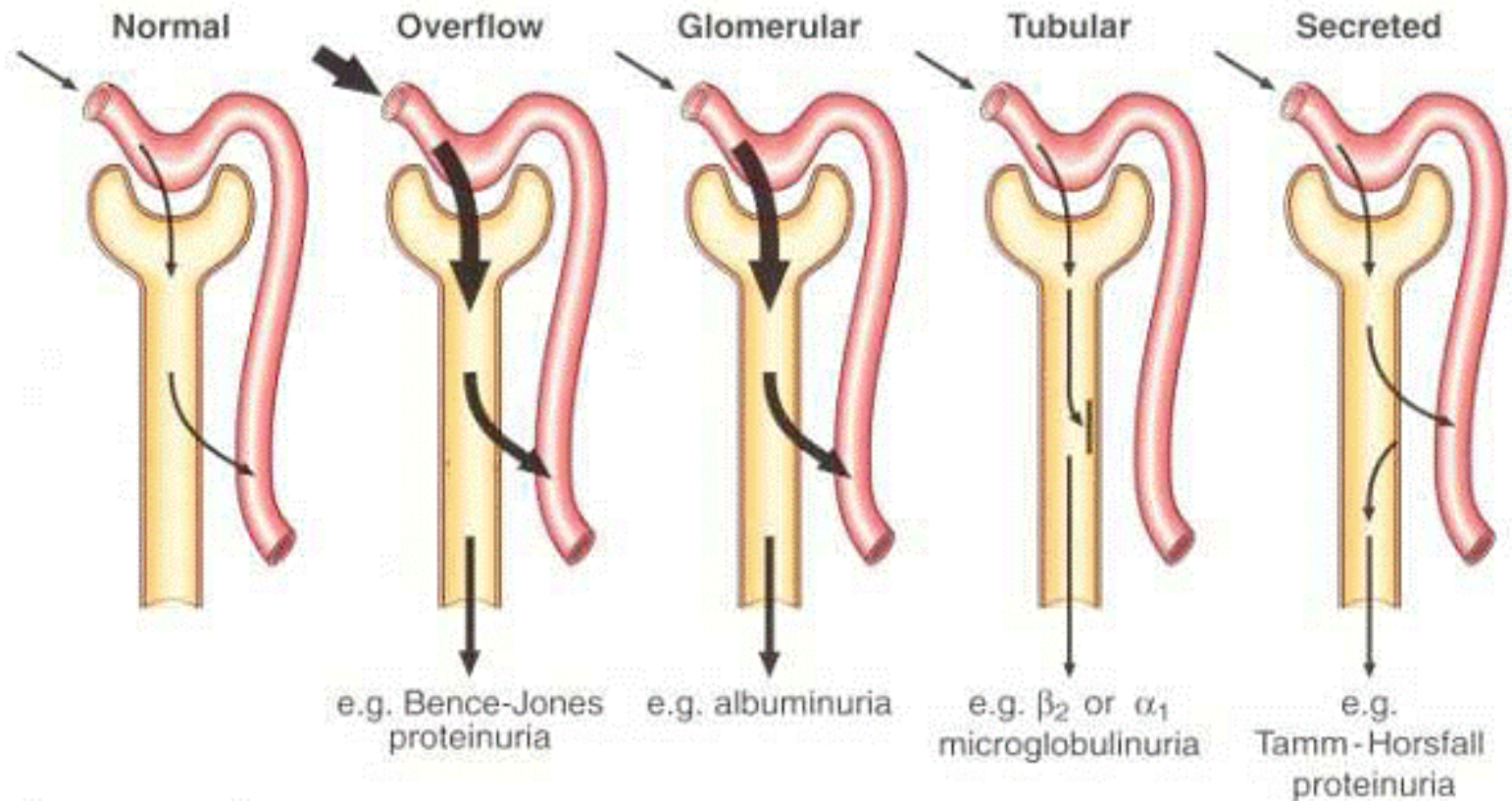


Fig. 1 The classification of proteinuria.

CAUSES OF PROTEINURIA AS RELATED TO QUANTITY

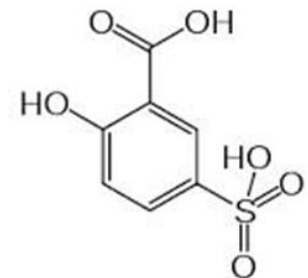
Protein amount per 24-hour	Type of proteinuria
0.15 to 2.0 g (150 -200 mg)	<ul style="list-style-type: none">• Tubular proteinuria• Overflow proteinuria (an increased proportion of low molecular weight proteins)
2.0 to 4.0 g (200-400 mg)	<ul style="list-style-type: none">• Usually glomerular
➤ > 4.0 g (more than 400 mg)	<ul style="list-style-type: none">• Always glomerular (mainly albumin)



- *The quantitative estimation* of the daily excretion of protein is of value to the clinician in order to give a general idea of the type of renal disease, its severity and to monitor the results of treatment given.
- The protein content can be determined by numerous methods eg, Biuret, Lowry, Bradford,.....
- *In this lab* turbidimetric method will be used
- *Determination of* total protein by measurement of protein turbidity produce by mixed with an anionic organic acid such as sulfosalicylic acid , TCA , or benzethonium chloride.
- *Sulphosalicylic acid is* used in this experiment to precipitate the protein in *a 24 hour* sample of urine. The turbidity is proportional to the concentration of the protein, and may be measured with a spectrophotometer at 500 nm.

SULFOSALICYLIC ACID (SSA) TEST:

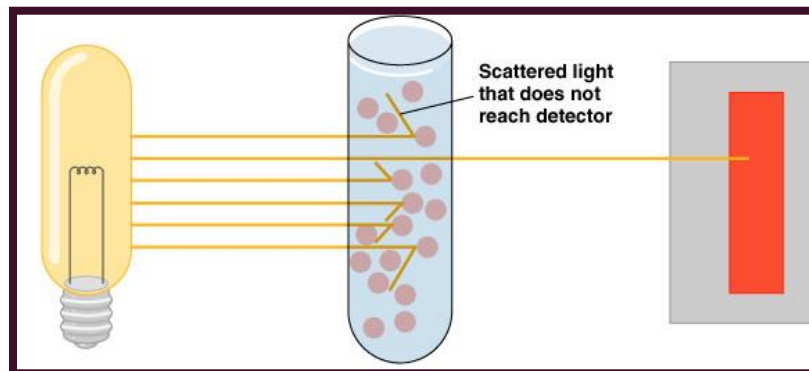
- *The sulfosalicylic acid (SSA)* turbidity test quantitatively screens for proteinuria. The advantage of this easily performed test is its greater sensitivity for proteins such as Bence Jones.
- The SSA reaction will detect globulin and Bence-Jones proteins, in addition to albumin (although it is more sensitive to albumin).



sulfosalicylic acid

PRINCIPLE

- Sulfosalicylic acid is an *anion(-)* which neutralizes the protein *cations(+)* leading to its precipitation (pH in highly acidic media, the protein will be positively charged, which is attracted to the acid anions that cause them to precipitate.)
- Then the radiation of a wavelength which is not absorbed by the solution is made to pass through the suspension and the apparent absorption will be solely because of the scattering by the particles.



METHOD:

- 1- Set up a series of test tube as follows, label from 1- 7

Tube	Protein Stock Solution(140 mg/dl)	0.85% Saline	Protein concentration mg/dl
1	4.5	1.5	
2	3	3	
3	2.4	3.6	
4	1.5	4.5	
5	0.9	5.1	
6	0.3	5.7	
7(Blank)	0	6	
Urine Sample	-	-	

- 2-Set another 8 test tube labeled 1-7 and pipette in each one Add 8 ml of sulfosalicylic acid



Tube	sulfosalicylic acid
1	8 ml
2	8 ml
3	8 ml
4	8 ml
5	8 ml
6	8 ml
7(Blank)	8 ml
Urine Sample	8 ml

METHOD

- 3-Into tube 1 pipette 2 ml of protein solution 1, into tube 2 pipette 2 ml of protein solution 2 ect. For the Urine Sample pipette 2ml of the Sample
- 4-Mix contents of each tube well and allow standing for 5 minutes.
- 5-Using solution 7 (Blank) to set transmittance at 100 at 500nm.
- 6-Then use solutions from 1-6, to recorded respective transmittance of each suspension.

Results:

Tube	Transmittance at 500 nm	Protein concentration mg/dl
7(Blank)	100 %	
1		
2		
3		
4		
5		
6		
Urine Sample		

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- Plot Transmittance against Protein concentration mg/dl
 - Read the Protein concentration of Urine Sample from the standard curve
 - Compare the result you got with the normal range of protein excretion in 24 h urine specimen if you know that the protein excretion in healthy sample (0- less than 0.150g/24 h). Comment on the clinical conditions of the patient if it is present..

REFERENCES

- The Washington Manual™: Nephrology by Steven Cheng, Anitha Vijayan, Katherine E. Henderson, and Thomas M. De Fer 3rd edition published by Wolters Kluwer (<https://www.inkling.com/read/washington-manual-nephrology-cheng-vijayan-3rd/chapter-4/approach-to-proteinuria>)
- A Manual of Laboratory and Diagnostic Tests 9th edition (January , 2014), Frances T Fischbach RN, BSN, MSN By Lippincott Williams & Wilkins Publishers
- » Clinical Biochemistry, An Illustrated Colour Text 4th edition, Allan Gaw, Michael J. Murphy, Robert A. Cowan, Denis St. J. O'Reilly, Michael J. Stewart, James Shepherd
- » BCH 472 BCH practical note

