Detection and Estimation of Some Abnormal Constituents

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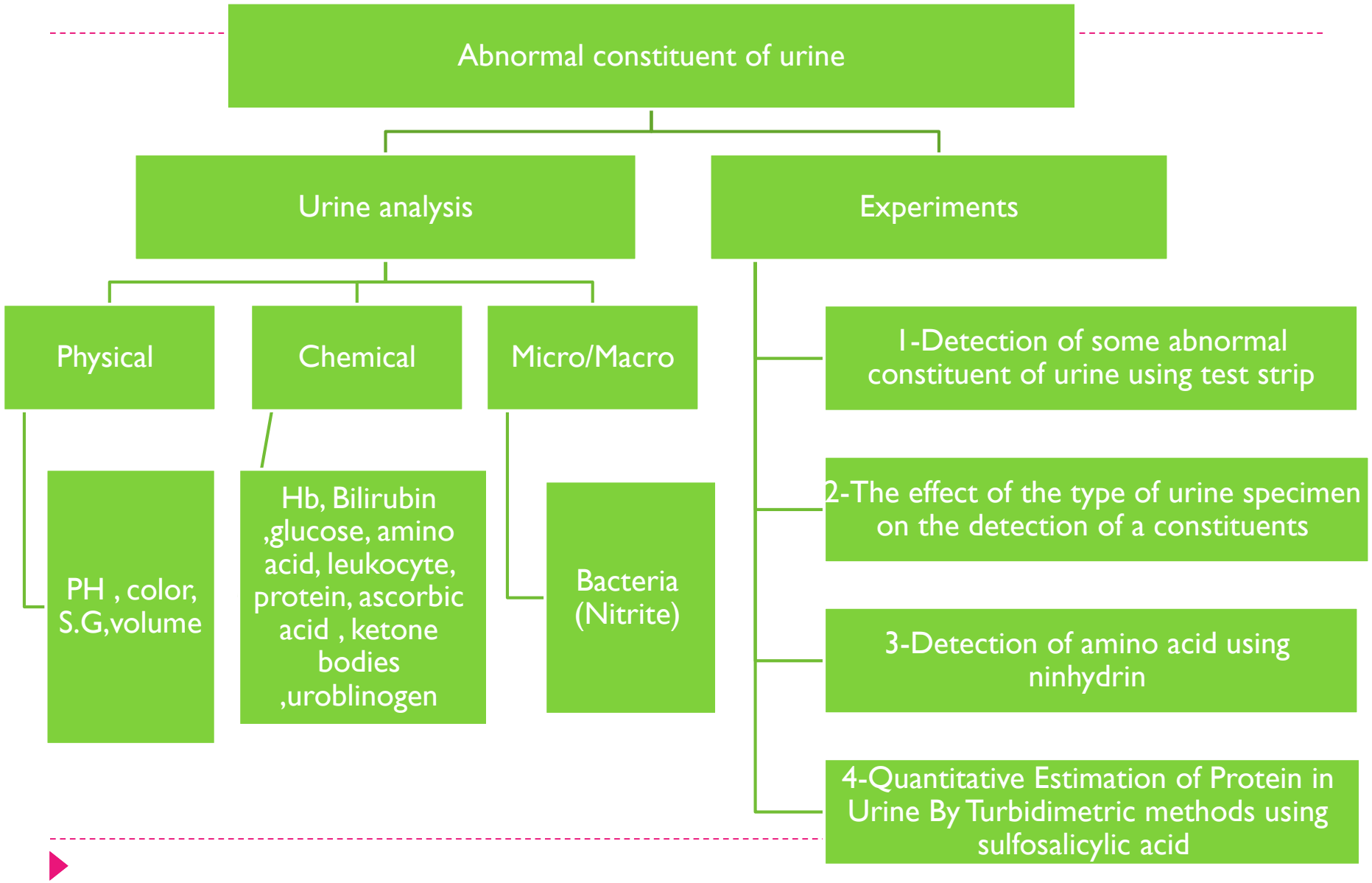
Lecture Over view



Physical Analysis:

The following are some abnormal constituent that not normally found in detectable amount:

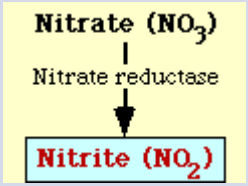
Positive in Urine	Obtained Results	Cause
PH	• **Acidic below 5**	• Diabetic ketoacidosis
	• **Alkaline above 8**	• Vegetarian diet • Strongly alkaline is due to bacteria infection
Color	• Dark yellow , Orange	• dehydrated • metabolic disorders • **Medications**
	• Pink or Red color	• Hematuria • **Medications**
Specific Gravity	High	• Diarrhea that causes dehydration • Sugar, or glucose, in the urine
	Low	• Damage tubule cells (renal tubular necrosis) • Diabetes insipidus
Volume	• Polyuria : > 2000ml/day • Oliguria : 500 – 200 ml/day • Anuria : 0 – 125 ml /day	• polyuria and oligouria observed in Physiological and Pathological condition • Anuria :Obstructive collected adduct due to a stone or tumor

Chemical Analysis:

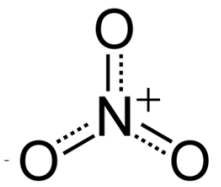
The following are some abnormal constituent that not normally found in detectable amount:

Positive in Urine	Cause	Notes
Blood (hematuria)	• Bleeding because of damage to kidney or genitourinary system, **eg**: **Renal Calculi, Renal Tumor, Truma to kidneys**	• Any pink, red or brown urine must be considered as bloody until proved otherwise.
Haemoglobinuria	• Is due to intravascular haemolysis.	
Leukocyte	• Urinary tract infection bacteria	
Ascorbic acid	• Large urinary concentrations arise from therapeutic doses of vitamin C.	

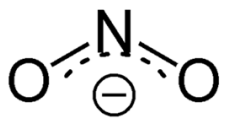
Chemical Analysis:

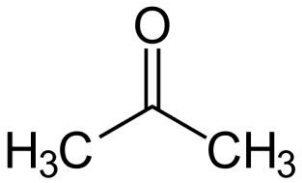
Positive in Urine	Cause	Note
Glucose (Glycosuria)	• Blood glucose level exceeds the reabsorption capacity of the tubules, **eg**, **Diabetes mellitus** • Defect in the tubular reabsorption eg.**fanconi syndrome**	Glucose is present in the glomeroular filtrate and reabsorbed by the proximal tubules
Ketone bodies	• Occur whenever increased amounts of metabolized fat **eg**, **Diabetes mellitus**, **Starvation**	• Urine may have a fruity (aceton) smell
Nitrite	• Urinary tract infection Bacteria that can reduce the nitrate to nitrite	 Bacteria that can reduce the nitrate to nitrite

NITRATE

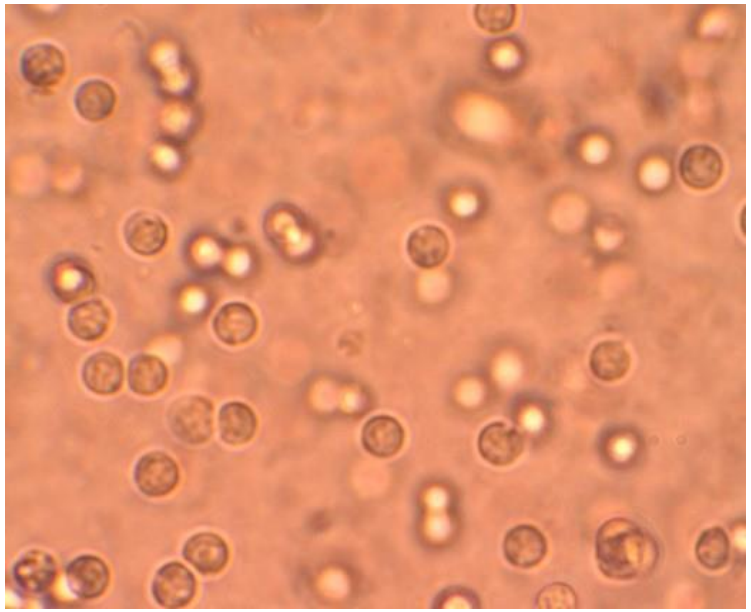


NITRITE



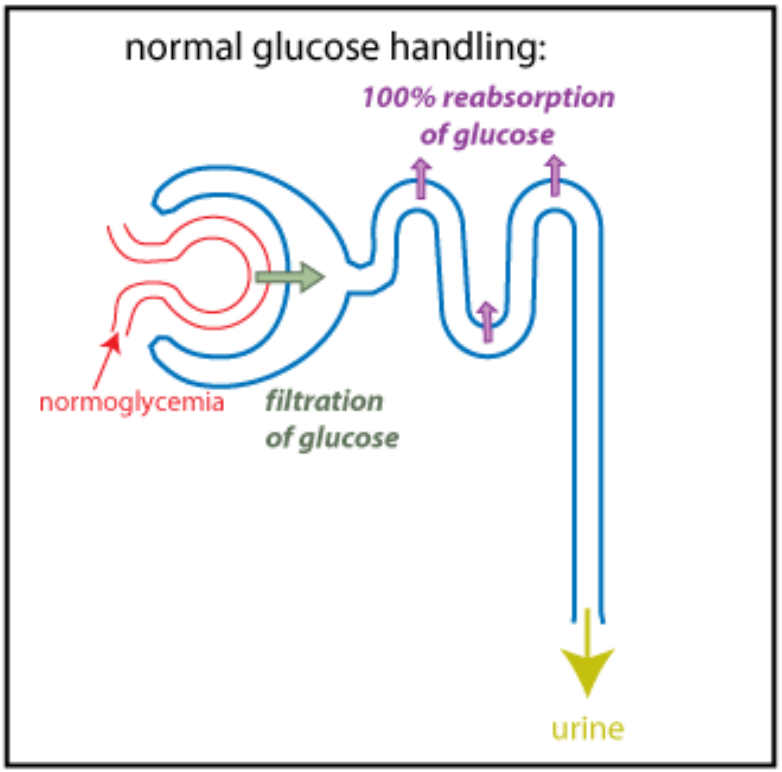


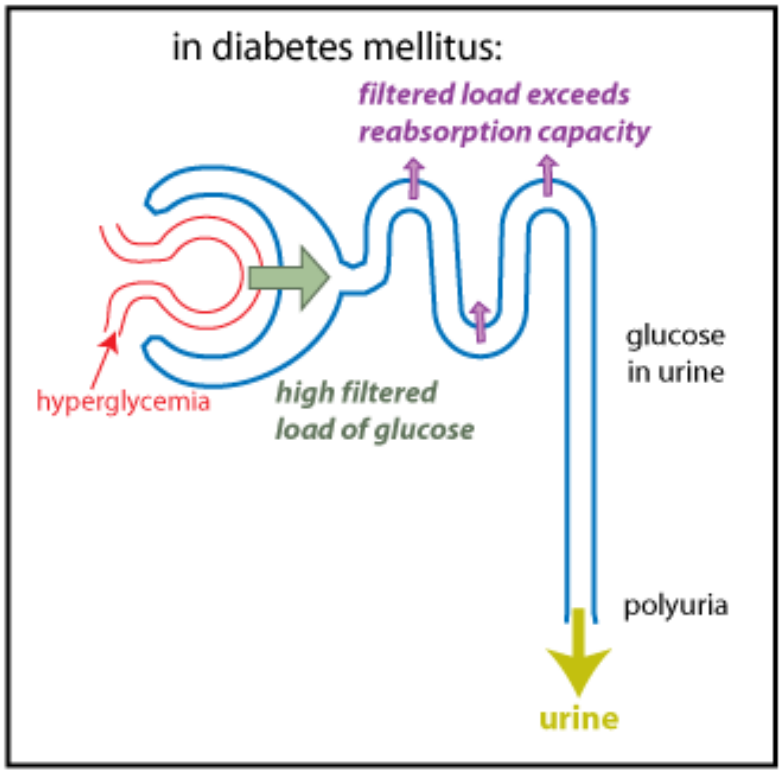
Ketone



Nitrate developing urinary tract infections. Urinary tract infections caused by bacteria are generally treated with correct antibiotics

Glucose filtration and reabsorbing





Chemical Analysis:

Positive in Urine	Cause	Note
Bilirubin	• Elevated amount of bilirubin in the blood stream, **eg**, **Bile duct obstruction**	• The urine may be dark with a yellow foam if much is present
Uroblinogen	• Increased production **eg, hemolytic jaundice**	• Its presence does not give a colored foam
Amino acid (aminoaciduria)	• Blood amino acid level exceeds the reabsorption capacity of the tubules **eg**, **Phenylketonuria**, **Alkaptouria** • Defect in the tubular reabsorption **eg**, **fanconi syndrome**, **cystinuria**	

Test strip (dipstick)

- ***The test strips consist of*** a ribbon made of absorbent microfiber cellulose pads attached to it.

- ***Each pad contains*** the dried reagents needed for a specific test that react with the compounds present in urine producing a characteristic colour. The depth of color produced relates to the concentration of the substance in the urine.

- ***It provides quick*** **Semi-quantitative** determinations of pH, protein, glucose, ketones, bilirubin, hemoglobin (blood), nitrite, leukocyte , urobilinogen, and specific gravity.

- ***Color changes then*** matched to the control chart at the correct time after each stick is dipped into the urine specimen.

Test strip (dipstick)

- ***Normally,*** substances such as nitrate, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are present in very small quantities that is not capable of detection by this method.
- but present in detectable amount are ***not normal***

False positive and false negative are common when using dipstick

	False-positive	False-negative
protein	Alkaline Urine Ammonia	Dilute Urine
Glucose	Strong Oxidizing agent	Ascorbic acid
Blood	Oxidizing contaminants	High Ascorbic acid
Bilirubin	Pigmented urine	Ascorbic acid,nitrite
Uroblinogen	Alkaline Urine	Nitrite formaline
Nitrite	Pigmented urine	Ascorbic acid
Leukocytes	Oxidizing detergent	

Protein in urine

- 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.
- The quantitative estimation of the daily excretion of protein is of value to the clinician in order to determine the type of renal disease, its severity and to monitor the results of treatment given.

Types of proteinuria

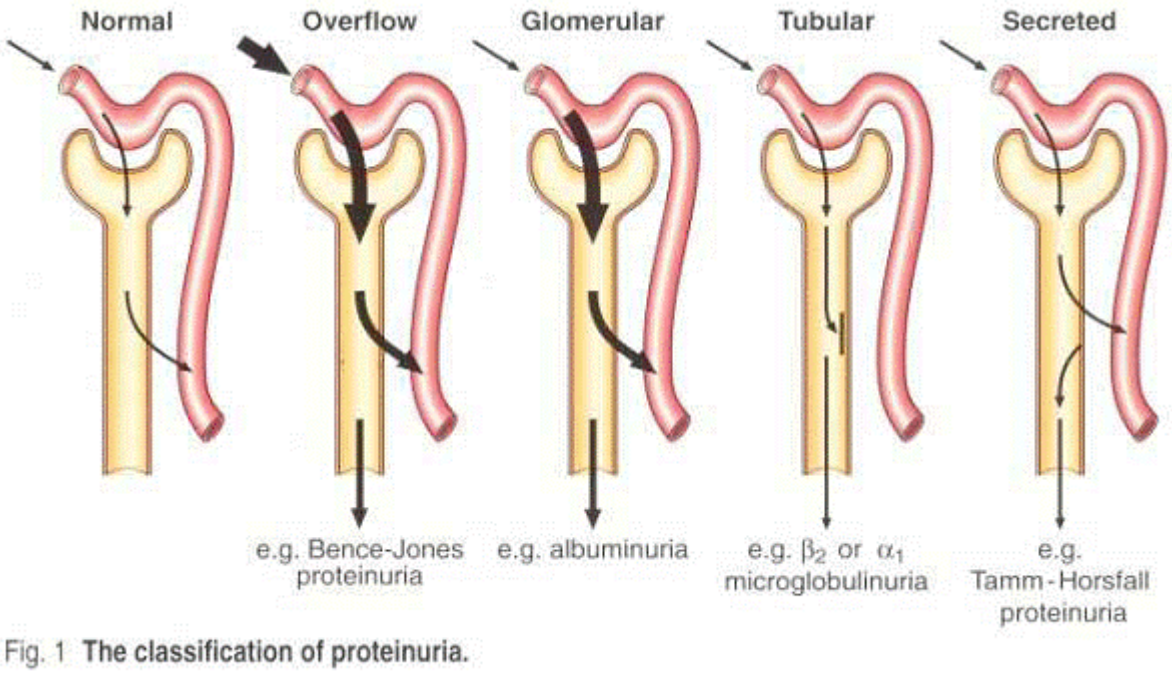


Fig. 1 **The classification of proteinuria.**

- **Glomerular proteinuria**
- has higher levels of protein , often exceeding 2 g /day ,mainly albumin
- **Tubular proteinuria**
- shows a moderate increase in urinary proteins , usually less than 2 g /day , and show an increased proportion of low molecular weight proteins

Types of proteinuria

Type	Cause
Glomerular proteinuria	Damage to the glomerular which increased filtration of normal plasma protein and because albumin has the highest concentrtion in the plasma it is called abuminuria eg. Malignant hypertention
Tubular proteinuria	Defect in the reabsorption(low molecular wight protein) eg, Fanconi Syndrom
Overflow proteinuria	overflow of high plasma concentrations of **low molecular weight protein** eg, Multiple myloma
Secretory proteinuria	oversecretion of certain proteins in the tubules, most notably the oversecretion of Tamm-Horsfall proteins in interstitial nephritis

Experiments:

1-Detection of some abnormal constituent of urine using test strip.

2-The effect of the type of urine collection on the detection of Urine constituents.

3-Detection of amino acid using ninhydrin

4-Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid

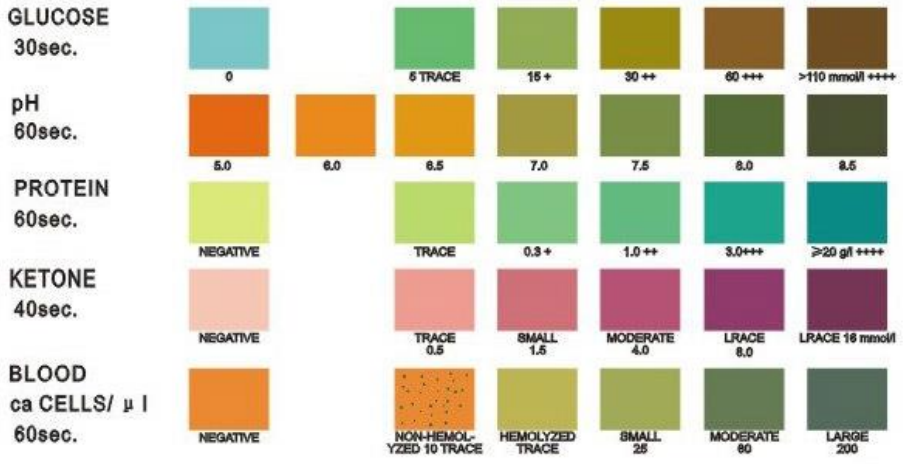
Objectives:

1- The semi-quantitative detection of some abnormal constituents by means of test-strips.

2- The detection of amino-acids in abnormal urine.

3- To investigate the difference between urine specimen

4-The quantitative estimation of protein in abnormal urine.



1-Detection of some abnormal constituent of urine using test strip

- You will have 2 different urine sample:
- You should fill the following information and then the probable diagnosis:

Test Parameter	Results of Sample 1	Results of Sample 2
Volume		
Color		
pH		
Blood		
Bilirubin		
Uroblinogen		
Glucose		
Ketone		
Diagnosis		

2-The effect of the type of urine collection on the detection of Urine constituents

- You have two samples, one is random urine sample, the other is 24-hour urine sample from the same patient,
- Compare between the two samples using the test strip

Test Parameter	24 hour Urine sample	Random urine Sample
Protein		

3-Detection of amino acid using ninhydrin

Principle

- Ninhydrin reacts with all amino acids except proline and hydorxyproline at pH 3-4 to give a purple colored compound. Proline will give a yellow color
- Initially, the amino acid is oxidized to an aldehyde containing one carbon atom less together with the release of ammonia and carbon dioxide. Then the ammonia, ninhydrin and the reaction product hydrindantin react to form the purple product.

Method

- As standard, use proline and glycine :
- Label three test tubes, A, B, C then add the following:
- 1 ml of glycine solution in tube A
- 1 ml of proline solution in tube B
- 1 ml of Abnormal urine in tube C
- Add a few drops of ninhydrin solution to each test-urine.
- Boil the contents of each test tube for 2 minutes.
- Record your observations.

Solution	Observation
Glycine	
Proline	
Urine sample 3	

4-Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid

Principle :

- 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.

- Method:

As in lab sheet

References

- http://www.nlm.nih.gov/medlineplus/ency/article/003583.htm
- A Manual of Laboratory and Diagnostic Tests 7th edition (July 2003): By Frances T Fischbach RN, BSN, MSN By Lippincott Williams & Wilkins Publishers