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Manual of Practical Medical Biochemistry

As per the Competency Based Medical Education Curriculum (NMC)

Evangeline Jones

THIRD EDITION

JAYPEE

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Competency Table

Topic: Biochemistry Laboratory Tests		*Number of competencies (22)*		*Number of procedures that require certification (05)*			*Chapter/Page no.*	
Number	*Competency The student should be able to*	*Core (Y/N)*	*Suggested teaching-learning method*	*Suggested assessment method*	*No. Required to certify*	*Integration: Vertical/ Horizontal*	*Chapter*	*Page no.*
BI11.1	Describe commonly used laboratory apparatus and equipments, good safe laboratory practice and waste disposal.	Y	Lecture, small group discussion	Written/Viva voce			2	4
BI11.2	Describe the preparation of buffers and estimation of pH.	Y	Lecture, small group discussion	Written/Viva voce			3	13
BI11.3	Describe the chemical components of normal urine.	Y	Lecture, small group discussion	Written/Viva voce			5	21
BI11.4	Perform urine analysis to estimate and determine normal and abnormal constituents.	Y	DOAP session	Skill assessment	1	General medicine/ Physiology	6	28
BI11.5	Describe the use of paper chromatography.	Y	Lecture, small group discussion	Written/Viva voce		General medicine	25	105
BI11.6	Describe the principles of colorimetry.	Y	Lecture, small group discussion	Written/Viva voce			9	45
BI11.7	Demonstrate the estimation of serum creatinine and creatinine clearance.	Y	Practical	Skills assessment	1		13	62

(*Contd...*)

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(*Contd...*)

Number	*Competency The student should be able to*	*Core (Y/N)*	*Suggested teaching-learning method*	*Suggested assessment method*	*No. Required to certify*	*Integration: Vertical/ Horizontal*	*Chapter*	*Page No.*
BI11.8	Demonstrate estimation of serum proteins, albumin and AG ratio.	Y	Practical	Skills assessment	1		15	73
BI11.9	Demonstrate estimation of serum cholesterol and HDL cholesterol.	Y	Practical	Skills assessment			16	77
BI11.10	Demonstrate the estimation of triglycerides.	Y	Practical	Skills assessment			17	79
BI11.11	Demonstrate estimation of calcium and phosphorous.	Y	Practical	Skills assessment			18	82
BI11.12	Demonstrate the estimation of serum bilirubin.	Y	Practical	Skills assessment			19	87
BI11.13	Demonstrate the estimation of SGOT/SGPT.	Y	Practical	Skills assessment			20	90
BI11.14	Demonstrate the estimation of alkaline phosphatase.	Y	Practical	Skills assessment			21	92
BI11.15	Describe and discuss the composition CSF.	Y	Lecture, small group discussion	Written/Viva voce			8	41
BI11.16	Observe use of commonly used equipments/ techniques in biochemistry including: ▪ pH meter ▪ Paper chromatography of amino acid ▪ Protein electrophoresis ▪ TLC, PAGE ▪ Electrolyte analysis by ISE ▪ Autoanalyzer ▪ DNA isolation from blood/tissue	Y	Demonstration	Skill assessment		General medicine/ Pathology	4 24–33	16 103–128

(*Contd...*)

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(*Contd...*)

Number	*Competency The student should be able to*	*Core (Y/N)*	*Suggested teaching-learning method*	*Suggested assessment method*	*No. Required to certify*	*Integration: Vertical/ Horizontal*	*Chapter*	*Page No.*
BI11.17	Explain the basis and rationale of biochemical tests done in the following conditions: ▪ Diabetes mellitus ▪ Dyslipidemia ▪ Myocardial infarction ▪ Renal failure, gout ▪ Proteinuria ▪ Nephrotic syndrome ▪ Edema ▪ Jaundice ▪ Liver diseases, pancreatitis ▪ Acid-base disorders ▪ Thyroid disorders.	Y	Lecture, small group discussion	Written/Viva voce			11 14 22 23 35–40 43	51 68 94 101 137–152 165
BI11.18	Discuss the principles of Spectrophotometry.	Y	Lecture, small group discussion	Written/Viva voce			10	49
BI11.19	Outline the basic principles involved in the functioning of instruments commonly used in biochemistry laboratory and their applications.	Y	Lecture, small group discussion	Written/Viva voce			12 23–32	58 101–125
BI11.20	Identify abnormal constituents in urine, interpret the findings and correlate these with pathological states.	Y	DOAP sessions	Skill assessment	1		6 7	28 34
BI11.21	Demonstrate estimation of glucose, creatinine, urea and total protein in serum.	Y	DOAP sessions	Skill assessment	1		11–15	51–73

(*Contd...*)

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(*Contd...*)

Number	*Competency The student should be able to*	*Core (Y/N)*	*Suggested teaching-learning method*	*Suggested assessment method*	*No. Required to certify*	*Integration: Vertical/ Horizontal*	*Chapter*	*Page No.*
BI11.22	Calculate Albumin: Globulin (AG) ratio and creatinine clearance.	Y	Lecture, small group discussion	Written/Viva voce		General medicine	13 15	62 73
BI11.23	Calculate energy content of different food items, identify food items with high and low glycemic index and explain the importance of these in the diet.	Y	Lecture, small group discussion	Written/Viva voce		General medicine	41	157
BI11.24	Enumerate advantages and/ or disadvantages of use of unsaturated, saturated and trans fats in food.	Y	Lecture, small group discussion	Written/Viva voce		General medicine	42	160
PY4.8	Describe and discuss gastric function tests, pancreatic exocrine tests and liver function tests.	Y	Lecture, small group discussion	Written/Viva voce			34–36	133–144
PY7.8	Describe and discuss renal function tests.	Y	Lecture, small group discussion	Written/Viva voce			38	145
PY8.4	Describe function tests: Thyroid gland, adrenal cortex, adrenal medulla and pancreas	Y	Lecture, small group discussion	Written/Viva voce			39 40	148 152

DOAP session—Demonstrate, Observe, Assess, Perform

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CHAPTER

13

Estimation of Serum and Urine Creatinine: Creatinine Clearance

BI11.7	Demonstrate the estimation of serum creatinine and creatinine clearance.
BI11.22	Calculate albumin: Globulin (AG) ratio and creatinine clearance.

ESTIMATION OF CREATININE IN SERUM (BY ALKALINE PICRATE METHOD—BASED ON JAFFE'S REACTION)

A. By Alkaline Picrate Method—Based on Jaffe's Reaction

Creatinine reacts with alkaline picrate reagent to form a reddish orange colored complex known as Creatinine Picrate. This color is measured in colorimeter at 540 nm using green filter. The intensity of the color is directly proportional to the concentration of creatinine present in the sample.

Reagents

1. 10% Sodium tungstate
2. 2/3 N Sulfuric acid
3. 0.75 N Sodium hydroxide
4. 0.04 M Picric acid
5. Creatinine standard:
   a. Stock standard: 100 mg% (100 mg in 100 mL of N/10 HCl)
   b. Working standard: 4 mg% (4 mL of stock diluted to 100 mL).

Procedure

A. Preparation of Protein-free Filtrate

Take 3 centrifuge tubes and label them as Blank (B), Test (T) and Standard (S) and proceed as follows:

Reagents	*Blank (B)*	*Standard (S)*	*Test (T)*
Distilled water	4 mL	3.5 mL	3.5 mL
Serum	—	—	0.5 mL
Working standard	—	0.5 mL	—
10% Sodium tungstate	0.5 mL	0.5 mL	0.5 mL
2/3 N Sulfuric acid	0.5 mL	0.5 mL	0.5 mL
Mix well. Wait for 10 minutes. Centrifuge the tube labeled T.			

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B. Development of Color (Creatinine Picrate)

Take 3 long test tubes and label them as Blank (B), Standard (S) and Test (T).

To the tube labeled standard (S), pipette out 3 mL of diluted working standard solution from the centrifuge tube labeled (S). To the tube (T) pipette out 3 mL of protein-free filtrate from the centrifuge tube labeled (T). To the blank tube (B), pipette out 3 mL of solution from the centrifuge tube labeled (B). Arrange the 3 tubes in a rack, and add 1 mL of picric acid and 1 mL of 0.75 N NaOH to all the tubes. Mix well. Wait for 10 minutes at room temperature. Take reading in colorimeter at 540 nm using green filter.

Reagents	*Blank (B) mL*	*Standard (S) mL*	*Test (T) mL*
Filtrate (Blank)	3	—	—
Filtrate (S)	—	3	—
Filtrate (T)	—	—	3
Picric acid	1	1	1
0.75N NaOH	1	1	1

Calculation

Concentration of creatinine in 100 mL of serum

$$= \frac{ODT - ODB}{ODS - ODB} \times \frac{\text{Concentration of Std}}{\text{Actual Volume of Blood}} \times 100 \text{ mg}$$

$$= \frac{T - B}{S - B} \times \frac{0.012}{0.3} \times 100 \text{ mg}$$

$$= \frac{T - B}{S - B} \times \frac{12}{3} \text{ mg / dL}$$

$$= \frac{T - B}{S - B} \times 4 \text{ mg / dL}$$

Conc. of Standard—Creatinine

- Stock standard Conc.: 100 mg%
- Working standard: 4 mg%
- 100 mL contains: 4 mg%
- Hence, 0.5 mL of standard contains: 0.02 mg%
- Total amount of filtrate: 5.0 mL
- 5.0 mL of filtrate contains: 0.02 mg
- Hence, 3 mL of filtrate contains: 0.02 × 3/5 = 0.06/5 = 0.012 mg.

Actual Volume of Blood

- Actual volume of blood taken: 0.5 mL
- 5.0 mL of filtrate is from: 0.5 mL blood
- Hence, 3 mL of filtrate contains: 0.5 × 3/5 = 0.3 mL blood

$$\frac{\text{Conc. of Standard}}{\text{Actual Volume of Blood}} \times 100 = \frac{0.012}{0.3} \times 100 = \frac{1.2}{0.3} = 4$$

Result: Creatinine present in 100 mL of serum/plasma = ...mg %

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ESTIMATION OF CREATININE (USING PROTEIN-FREE FILTRATE) (BY ALKALINE PICRATE METHOD—BASED ON JAFFE'S METHOD)

Principle

Creatinine reacts with alkaline picrate reagent to form a reddish orange colored complex known as creatinine picrate. This color is measured in colorimeter at 540 nm - green filter. The intensity of the color is directly proportional to the concentration of creatinine present in the sample.

Reagents

1. Protein-free filtrate
2. 0.75 N sodium hydroxide
3. Standard picric acid
4. Stock standard: 100 mg% in N/10 HCl
5. Working standard: 4 mg% (4 mL of stock diluted to 100 mL with N/10 HCl).

Procedure

Take three long test tubes. Mark them as Blank (B), Standard (S) and Test (T)

Reagents	*Blank (B)*	*Standard (S)*	*Test (T)*
Distilled water	3.0	—	—
Protein-free filtrate	—	—	3.0
Working standard	—	3.0	—
Saturated picric acid	1.0	1.0	1.0
0.75 N NaOH	1.0	1.0	1.0

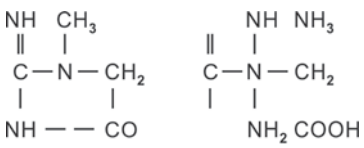
Calculation

Concentration of creatinine in 100 mL of the given sample

$$= \frac{OD(T) - OD(B)}{OD(S) - OD(B)} \times 4 \text{ mg/dL}$$

$$= \frac{T - B}{S - B} \times 4 \text{ mg\%}$$

Notes :
Normal level of creatinine
Serum/Plasma: 0.6–1.2 mg/100 mL
Urine: 1.0–2.0 g/24 hrs



Creatinine Creatine

Creatinine is the anhydride of creatine. Creatine is synthesized from 3 amino acids namely glycine, arginine and methionine. This occurs in 3 sites such as kidney, liver and skeletal muscles **(Fig. 13.1)**.

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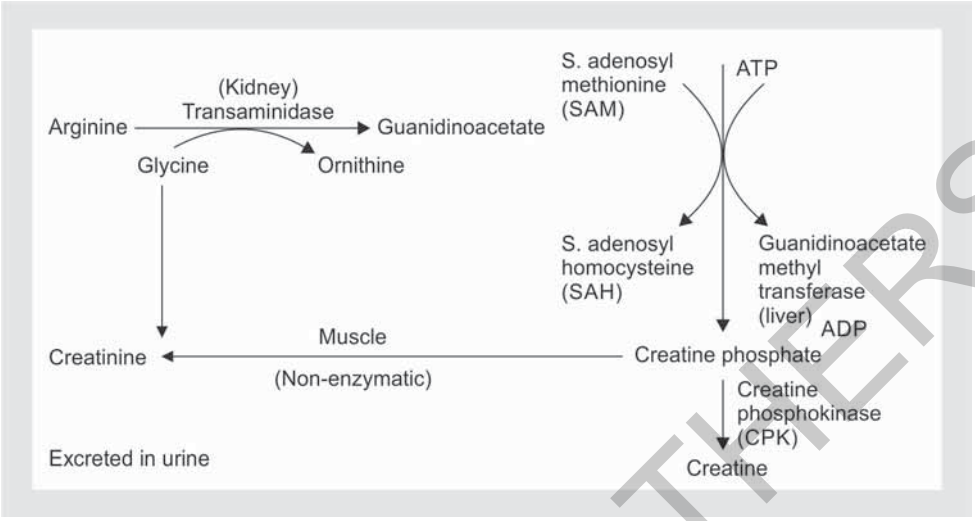

Fig. 13.1: Synthesis of creatinine.

Creatine phosphate (phosphagen) is the stored form of energy in muscle. About 2% of body creatine is daily excreted in urine as creatinine which is about 1–2 g/day for adults.

Creatinine Clearance

This is the most sensitive method of assessing glomerular function. Creatinine is filtered fully by the glomerulus but partially reabsorbed by the tubules of the kidney.

$$\text{Creatinine clearance} = \frac{U \times V}{P}$$

U = Concentration of creatinine in urine (mg/100 mL)
P = Plasma creatinine concentration (mg/100 mL)
V = Urine flow in one minute (mL/min)

Normal level:
- Males: 85–125 mL/min.
- Females: 80–115 mL/min

Clinical Interpretation

Increased level of creatinine in plasma is found in:
1. Renal diseases.
2. Congestive heart failure.
3. Obstruction of urinary tract.

Decreased creatinine clearance is a sensitive indicator of reduced glomerular filtration rate.

Other Methods of Estimation of Creatinine

By using Lloyd's reagent-hydrated aluminium silicate: *Corynebacterium ureafaciens* (NC-Bacteria) removes creatine and then Lloyd's reagent is used to absorb the creatinine and alkaline picrate is then added to produce the color.

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Relevant Questions

1. What is creatinine?
2. What is the relationship between creatine and creatinine?
3. How is creatinine synthesized?
4. Where is creatinine synthesized?
5. What is the normal serum creatinine level?
6. How much of creatinine is excreted normally in urine per day?
7. Name the conditions in which creatinine levels will be increased?
8. What is creatinine clearance? What is its normal value?

CREATININE IN URINE AND CREATININE CLEARANCE

Creatinine is one of the normal constituents of urine. Daily excretion of creatinine in urine ranges from 1 to 2 g. On a normal diet, almost all the creatinine in the urine is endogenous. It is derived from muscle creatine and is the anhydride of creatine.
It is increased in fever and myopathy.

Estimation of Creatinine in Urine (Jaffe's Reaction)

Principle: Creatinine in urine reacts with picric acid in the presence of sodium hydroxide to give an orange color (Jaffe's reaction). The color is measured at 500 nm.

Method: Dilute 1.0 mL urine to 100 mL with water in a standard flask. Therefore 5 mL of the diluted urine contains 0.05 mL urine.

Mark three test tubes Standard (S), Test (T) and Blank (B). Proceed as follows:

Reagent	*Std (mL)*	*Test (mL)*	*Blank (mL)*
Creatinine	5.0	—	—
Working std			
Diluted urine	—	5.0	—
Distilled water	—	—	5.0

Add to each tube, 1 mL of 1% picric acid followed by 1.0 mL of 0.75 N NaOH, mixing after each addition. Stand for 15 minutes for color development. Set blank to 0 in a photometer at 500 nm (Yellow green filter). Read OD of the blank, standard and test.

Calculations

$$\text{Creatinine in 0.05 mL urine} = \frac{\text{ODT} - \text{ODB}}{\text{ODS} - \text{ODB}} \times 0.05 \text{ mg}$$

$$\text{Creatinine in 100 mL urine} = \frac{\text{ODT} - \text{ODB}}{\text{ODS} - \text{ODB}} \times \frac{0.05}{0.05} \times 100 \text{ mg}$$

$$= \frac{\text{ODT} - \text{ODB}}{\text{ODS} - \text{ODB}} \times 100 \text{ mg}$$

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Creatinine Clearance

This is the most sensitive method of assessing glomerular function. Creatinine is filtered fully by the glomerulus but partially reabsorbed by the tubules of the kidney. It is defined as the number of mL of plasma cleared of creatinine per minute by the kidney.

$$\text{Creatinine clearance} = \frac{U \times V}{P}$$

U = Concentration of creatinine in urine (mg/100 mL)
P = Plasma creatinine concentration (mg/100 mL)
V = Urine flow in 1 minute (mL/min)

Normal level:
Males: 85–125 mL/min.
Females: 80–115 mL/min.

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Manual of Practical Medical Biochemistry

Salient Features

- This edition of *Manual of Practical Medical Biochemistry* comes with the compactness of the earlier editions incorporating the changes based on the Competency based Undergraduate Curriculum issued by the MCI/NMC
- Layout has been made student-friendly with a clear focus on clinical aspects of the art of practical medical biochemistry
- Latest equipments, laboratory procedures, requirements and clinical interpretations have been added
- Clinical Case studies and Objective Structured Practical Examination (OSPE) and other integrated topics have been updated in keeping up with the trend
- Clarity of figures and flowcharts make this Manual, easy to understand.

Evangeline Jones MD was Professor and Head of the Department of Biochemistry, Government Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, India for more than 15 years and then as Dean, Kanyakumari Government Medical College, Nagercoil, Tamil Nadu. Subsequently, she worked as Professor and Head, Department of Biochemistry, VMKV Medical College, Salem, where she was involved in teaching undergraduate and postgraduate students in Biochemistry, besides being a research guide for many. She had been the Chief Editor of the National Journal of Basic Medical Sciences for three years and has several research publications to her credit. She is the recipient of Lifetime Achievement Award from The Tamil Nadu Dr MGR Medical University, Chennai, for her excellence in teaching career.

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