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Competency-based Comprehensive Manual of Practical and Clinical BIOCHEMISTRY

As per the Revised Competency-based Medical Education Curriculum (NMC)

3rd Edition

Ashish Sharma
Anita Sharma

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Contents

UNIT 1: INTRODUCTION OF CLINICAL BIOCHEMISTRY

1.1. Laboratory Apparatus and Equipment, Good and Safe Laboratory Practice, and Waste Disposal Systems in Laboratory 3

UNIT 2: QUALITATIVE EXPERIMENTS AND THEIR CLINICAL APPLICATIONS

2.1. Analysis of Carbohydrates 13
2.2. Analysis of Proteins 28
2.3. Analysis of Physical and Chemical Composition of Physiological Urine 44
2.4. Identify, Perform and Interpret Pathological Urine Analysis and Correlate it with Pathological States 52

UNIT 3: QUANTITATIVE EXPERIMENTS AND THEIR CLINICAL INTERPRETATION

3.1. Principle of Colorimetry 65
3.2. Principle of Spectrophotometry 70
3.3. Estimation of Blood Glucose 76
3.4. Glucose Tolerance Test and Glycated Hemoglobin 84
3.5. Liver Function Test 90
3.6. Kidney Function Test 113
3.7. Lipid Profile (Atherogenic Profile) 124
3.8. Estimation of Serum Calcium and Serum Phosphorus 134

UNIT 4: SELF-DIRECTED LEARNING EXERCISES

4.1. pH Meter 141
4.2. Water Homeostasis and Estimation of Na^+ and K^+ with ISE Analyzer 144
4.3. Arterial Blood Gas Analyzer 147
4.4. Chromatography 151
4.5. Electrophoresis 157
4.6. Enzyme-linked Immunosorbent Assay 161
4.7. Antigen-Antibody Interaction (Immunodiffusion) 165
4.8. Quality Control in Clinical Laboratory 171
4.9. DNA Isolation from Blood and Tissue 176

UNIT 5: EARLY CLINICAL EXPOSURE EXERCISES AND REFLECTIVE WRITING

5.1. Analysis of Cerebrospinal Fluid 181
5.2. Thyroid Function Test 186
5.3. Pancreatic Function Tests 192
5.4. Disorders of Acid-Base Balance 197

UNIT 6: ATTITUDE, ETHICS, AND COMMUNICATION (AETCOM) MODULES

6.1. Introduction of Clinical Methods 205
6.2. What does it Mean to be a Doctor? 207
6.3. What does it Mean to be a Patient? 208
6.4. The Doctor-Patient Relationship 210
6.5 The Foundations of Communications 214

UNIT 7: BIOCHEMICAL CALCULATIONS AND REFERENCE RANGE

7.1. Preparations of Buffers and Solutions 219
7.2. Reference Value of Various Biochemical Parameters Integration with Medicine 221

UNIT 8: PRACTICAL SPOTS IN BIOCHEMISTRY

8.1. Spots 227

UNIT 9: BLUEPRINT IN BIOCHEMISTRY PRACTICAL ASSESSMENT

9.1. Blueprint in Biochemistry Practical Assessment 239

UNIT 10: LOGBOOK

10.1. Logbook 243

Index 259

2 UNIT

Qualitative Experiments and their Clinical Applications

OUTLINE

2.1. Analysis of Carbohydrates
2.2. Analysis of Proteins
2.3. Analysis of Physical and Chemical Composition of Physiological Urine
2.4. Identify, Perform and Interpret Pathological Urine Analysis and Correlate it with Pathological States

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2.1 Analysis of Carbohydrates

LEARNING OBJECTIVES

At the end of the session, the student must be able to:

- Differentiate between different types of carbohydrate important in health and diseases
- Detect different types of carbohydrate by qualitative analysis
- Detect unknown carbohydrate present in solutions
- Interpret different quantitative laboratory investigations related to disorders of carbohydrate metabolism

BC3.1: Discuss and differentiate monosaccharides, disaccharides and polysaccharides with examples, their importance as energy fuel, structural element, and storage molecule in human body.
Domain: Knows.
Level: Knows how.
Core competency: Yes.

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INTRODUCTION

Carbohydrates are defined as polyhydroxy aldehydes or ketones or their derivatives. They are classified into monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

The following carbohydrates are generally given for practical exercises: glucose, fructose, maltose, lactose, sucrose, starch, and dextrin.

CLASSIFICATION

Broadly classified as:

- **Monosaccharides:** Simplest sugars, which cannot be hydrolyzed further into smaller compounds, e.g. glucose, fructose.
- **Disaccharides:** They are formed by condensation of two monosaccharide molecules, which may be similar or dissimilar, e.g. maltose, lactose, and sucrose.
- **Polysaccharides:** They are formed by condensation of large numbers of monosaccharide molecules. On hydrolysis, they give more than ten monosaccharide units, which may be similar or dissimilar, e.g. starch, glycogen, heparin, hyaluronic acid, etc.

MOLISCH'S TEST

This is the group test for carbohydrates given positive by all carbohydrates whether free or bound to such substances as protein (glycoprotein and mucoprotein) and lipids (glycolipids) **(Fig. 2.1.1)**.

Experiment	*Observation*	*Inference*
Molisch's test: 2 mL of original solution + 2 drops of 1% alcoholic α-naphthol (Molisch's reagent). Mix and add 2 mL of concentrated H_2SO_4 slowly from the side of the test tube	Purple ring at the junction of the two liquids	*Carbohydrates present:* It is a general test, which indicates the presence of carbohydrates

Principle

Carbohydrates undergo dehydration when treated with concentrated H_2SO_4 to form a five-membered cyclic

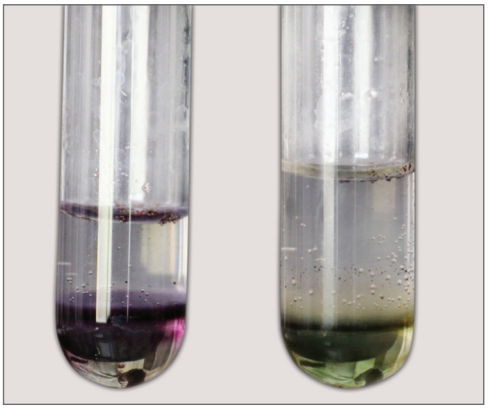

Fig. 2.1.1: Molisch's test.

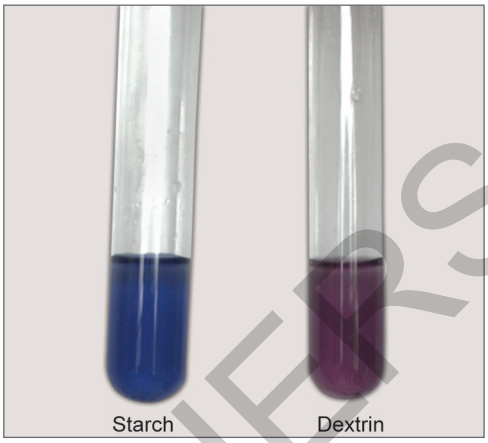

Fig. 2.1.2: Iodine test.

compound, furfural or its derivative, which on condensation with α-naphthol forms a purple-colored complex.

Fallacies

Furfural, aldehydes, and some organic acids, e.g. formic acid, oxalic acid, lactic acid, citric acid, etc. also give a positive Molisch's test **(Fig. 2.1.1)**.

Precautions

- Test tube must be dry.
- Do not shake the test tube to get a positive test.

IODINE TEST

This is the test for polysaccharides. Starch, dextrin, and glycogen can be differentiated by this test.

Experiment	*Observation*			*Inference*
3 mL of original solution + 2 drops of iodine solution Mix.	No color develops			Polysaccharides absent
	Color	On heating	On cooling	
	Blue	Disappears	Reappears	Starch
	Reddish purple	Disappears	Reappears	Dextrin
	Reddish brown	Disappears	Reappears	Glycogen

Principle

The test depends upon the property of adsorption possessed by the large polysaccharide molecules, which adsorb the smaller iodine molecules on their surface to form a complex of undefined chemical nature. The starch-iodine, dextrin-iodine, and glycogen-iodine complexes are blue, reddish purple, and reddish brown, respectively. The adsorption is a physical property, which decreases on heating, the complex dissociates and color disappears. On cooling, the complex is reformed, and color reappears, except in the case of dextrin in which heating produces a nearly irreversible change, as a result it loses the property of adsorption. Monosaccharides and disaccharides do not produce a change in color **(Fig. 2.1.2)**.

Precautions

- The test should not be carried out in alkaline medium as iodine is not freely available and iodine adsorption does not occur.
- Excess of iodine should be avoided.
- To prevent hydrolysis, the solution must not be heated highly.

TEST FOR THE REDUCING SUGARS

The carbohydrates, which have a free aldehyde and ketonic groups, have the ability to reduce the solutions of various metallic ions.

Requirement

- *Reducing agent:* Carbohydrate having free aldehyde and ketone groups.
- Oxidizing agents having metallic ions, e.g. Cu^{+}, Cu^{2+}, Ag^{+}, etc. can be used as salt.
- *Medium:* Alkaline medium for Fehling's and Benedict's test. Acidic medium for Barfoed's test.
- Heating agent.

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Benedict's Test

This is a test for the reducing carbohydrates **(Fig. 2.1.3)**.

Test	*Observations*	*Inference*
Benedict's test: 5 mL of Benedict's reagent + 8 drops of original solution. Boil it for 2 minutes and allow it to cool	The color of the precipitate could be green/yellow/ orange/red/brick red depending on the concentration of reducing sugar in the solution	–

Principle

In the alkaline medium, cupric ions of the Benedict's solution are reduced to cuprous ions by the reducing sugars, which ultimately form Cu_2O as a red precipitate.

Advantages

- The reagent is very stable and can be stored in a single bottle for a long time.
- The test is convenient and simple.
- Since, sodium carbonate is a very weak base, the carbohydrates are not altered.
- This is **semi-quantitative** test. If the solutions are taken in proper proportion and procedure is followed strictly, the approximate concentration can be judged from the color of precipitation.

Grades	*Color of precipitation*	*Approximate concentration of carbohydrates*
0	Blue	No sugar
(+)	Green	0.1–0.5%
(++)	Yellow	0.5–1.0%
(+++)	Orange	1.0–2.0%
(++++)	Red	Above 2.0%

Practical Application

Detection of reducing sugar in urine.
Lactosuria—Lactose
Glucosuria—Glucose
Galactosuria—Galactose
Pentosuria—Pentose
Fructosuria—Fructose

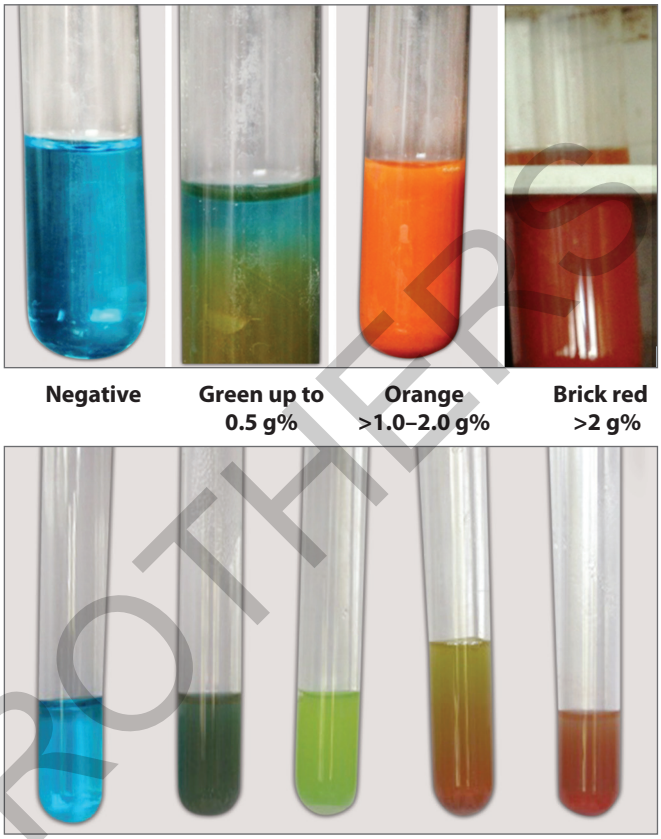

Fig. 2.1.3: Benedict's test.

Disadvantages

- Many substances with reducing groups react with cupric ion in alkaline medium at boiling temperature to form red precipitates of cuprous oxide.
  Noncarbohydrates giving false positive Benedict's test:
  - High concentration of uric acid, creatinine, and ketones
  - Homogentisic acid (solution turns black due to black-colored oxidized homogentisic acid)
  - Vitamin C (even without boiling)
  - Certain drugs like aspirin, cephalosporins.
- Reagent is unstable. It has to be prepared in 2 parts, which are stored separately.
- The strong alkali as KOH is present in the reagent, which can destroy carbohydrate.
- Autoreduction may occur resulting in the false-positive test.

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Exercise 2.1.1: Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Date: **Teacher's Signature**

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Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Date: **Teacher's Signature**

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Barfoed's Test

This test is for strong reducing monosaccharide **(Fig. 2.1.4)**.

Test	*Observation*	*Inference*
2 mL of Barfoed's reagent + 2 mL of original solution. Boil it for 30 seconds and allow it to cool	Scanty red precipitate sticking to the side and bottom of the test tube	Monosaccharide present. This test differentiates between monosaccharides and disaccharides. Disaccharides give this test negative

Principle

The reduction of cupric ions is carried out in acidic medium. Since acidic medium is unfavorable for reduction, only the strong reducing carbohydrates, i.e. monosaccharide give the test positive.

Precautions

1. Boiling period should not exceed 2 minutes otherwise disaccharide will hydrolyze and give positive test.
2. Chloride ions interfere with this test therefore, the test should not be carried out with solution containing chloride, i.e. urine.

Seliwanoff's Test

This test is for ketohexoses, i.e. fructose **(Fig. 2.1.5)**.

Test	*Observation*	*Inference*
3 mL reagent + 1 mL carbohydrate solution. Boil for 30 seconds and allow it to cool	Cherry-red color	Ketose sugar present. The test distinguishes between aldose and ketose sugars

Principle

The carbohydrates are converted into furfural derivatives by HCl, which condense with resorcinol to form cherry red or pink color complex.

Precautions

- Boiling should not exceed more than 60 seconds as prolonged boiling converts aldohexose to ketohexose giving false-positive test.
- The final concentration of HCl in Seliwanoff's reagent should not exceed 12% as strong acids convert aldohexoses to ketohexoses giving a positive test.

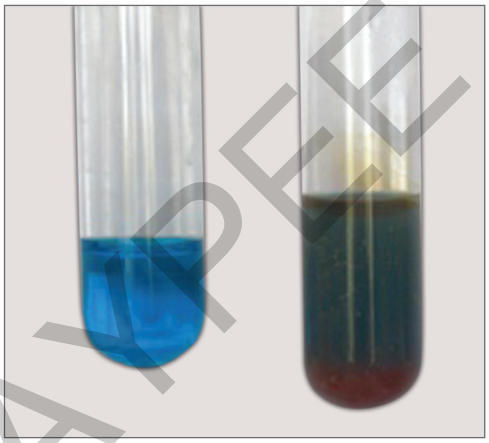

Fig. 2.1.4: Barfoed's test.

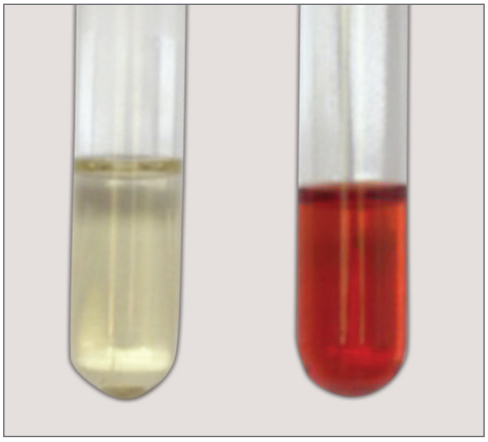

Fig. 2.1.5: Seliwanoff's test.

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Exercise 2.1.2: Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Date: **Teacher's Signature**

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Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Osazone Test

This is a test for the identification of reducing disaccharide and many monosaccharides by characteristic crystal formation. It is a confirmatory test **(Fig. 2.1.6)**.

Test	*Observation*	*Inference*	
Take 5 mL of glucose, fructose, maltose, and lactose solution in labeled test tube. Add 0.3 g of Osazone mixture + 5 drops of acetic acid. Put all test tubes in water bath	Yellow crystals will appear in the tubes containing glucose and fructose within 5 minutes and maltose and lactose after 45 minutes. Put crystal on slide and observe under microscope	Glucose	Needle shape
		Fructose	Needle shape
		Maltose	Sunflower
		Lactose	Puff shaped

Principle

When the reducing carbohydrates are treated with phenylhydrazine at 100°C and pH of 4.3, the reaction occurs at first and second carbon atoms to form phenyl hydrazone crystals, which are of specific shapes.

Inversion Test

This is a test for nonreducing sugar sucrose.

Test	*Observation*	*Inference*
Inversion test for sucrose: 3 mL of original solution + 2–3 drops of concentrated HCl. Boil for 2 minutes. Cool, neutralize with 2–3 drops of 40% NaOH or sodium carbonate till effervescence ceases. Then, perform: **i. Benedict's test *and*** **ii. Seliwanoff's test**	Precipitate of green/ yellow/orange/brick red Cheery-red color	Sucrose when boiled with concentrated HCl undergoes hydrolysis to yield glucose and fructose, which being reducing sugars give Benedict's test positive. Ketohexose present

Principle

When sucrose is boiled with concentrated HCl, it is hydrolyzed to form monosaccharide units of glucose and fructose.

$$\underset{+66.5°}{\text{Sucrose}} \longrightarrow \underset{-92.3°}{\text{Fructose}} + \underset{+52.5°}{\text{Glucose}}$$

The optical rotation changes from dextrorotatory to levorotatory as fructose possess a much stronger levorotation than dextrorotation caused by glucose. The change in optical rotation is, thus, known as **inversion**. So, sucrose, a mixture of glucose and fructose, is also called as **invert sugar**.

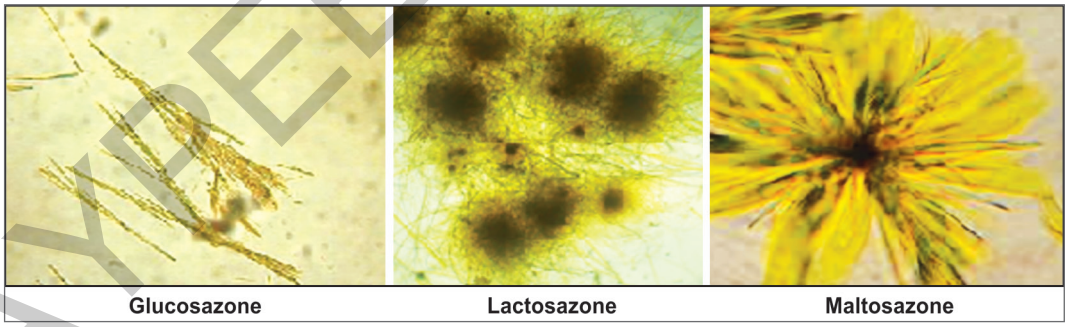

Fig. 2.1.6: Shape of osazones under microscope.
Courtesy: DM Vasudevan. Textbook of Biochemistry for Medical Students, 8th edition. Jaypee Brothers Medical Publishers (P) Ltd: New Delhi; India.

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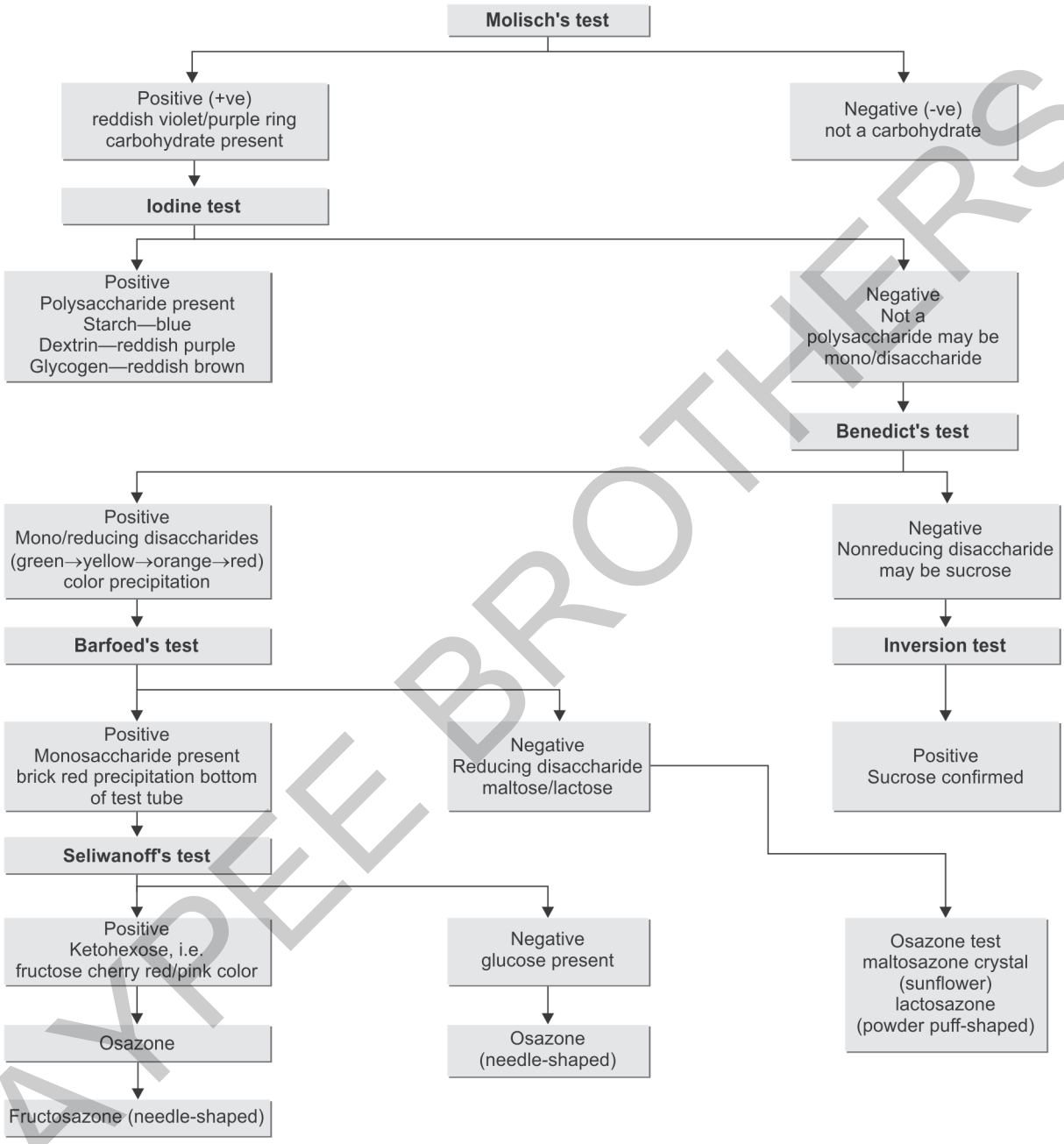
SCHEME FOR IDENTIFICATION OF UNKNOWN CARBOHYDRATE



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Exercise 2.1.3: Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Date: **Teacher's Signature**

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Analysis of carbohydrates.

Perform the following tests on sugar solution provided to you and record your observation and inference.

Test	*Observation*	*Inference*

Result and Interpretation

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Date: **Teacher's Signature**

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VIVA VOCE QUESTIONS: CARBOHYDRATES

1. Whether all carbohydrates give Molisch's test positive. Will glyceraldehydes 3-carbon aldose give Molisch's test positive?
2. Benedict's test is a semi-quantitative test. Why?
3. Describe different shape of crystal produced during osazone formation.
4. Name the typical carbohydrates found in the plants and animals.
5. Why on heating color disappears in starch-iodine solution and reappears on cooling?

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NOTES

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Competency-based Comprehensive Manual of

Practical and Clinical BIOCHEMISTRY

After the initiative of World Federation for Medical Education (WFME), it is recommended that Competency-based Medical Education (CBME) should be an integral part of curriculum of undergraduate medical students. Keeping this idea in mind, a complete and comprehensive practical and clinical biochemistry book based on Graduate Medical Regulations (GMR)-2019 is developed.

Salient Features

- Contains 10 sections, out of which 9 sections are inclusive of all core competencies of practical and clinical biochemistry and the last section is Biochemistry Logbook, essential for all necessary documentation as per updated CBME guidelines.
- Each chapter further explains specific competencies, learning objectives, level of learning and learning domain involved in clinical and practical biochemistry.
- *Basic laboratory principles:* It would enable students to know about apparatus and equipment used in the laboratory, good and safe laboratory practice and waste disposal system in laboratory, quality assurance and quality control in clinical biochemistry.
- Qualitative and quantitative experiments and their clinical interpretation with early clinical exposure including case studies and viva voce questions.
- Self-directed learning exercises.
- As spotting is integral part of practical examination "Practical Spots in Biochemistry" segment has been included.
- Biochemical calculations and reference range for various biochemical parameters.
- Competency-based assessment include blueprinting for biochemistry practical exams, certifiable competency as per National Medical Commission (NMC).
- Attitude and communication skill development exercise including clinical lab visits and reflective writing.

In the ever-changing and challenging fields of medicine, biochemistry is the mainstay in the basic development, diagnosis and the therapeutics of various human diseases. Hence, it is imperative to remain abreast of the latest development.

We hope this effort would enable the students to focus more in developing their knowledge of clinical interpretation rather than writing practical files and hence will upgrade their skills in practical and clinical biochemistry.

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