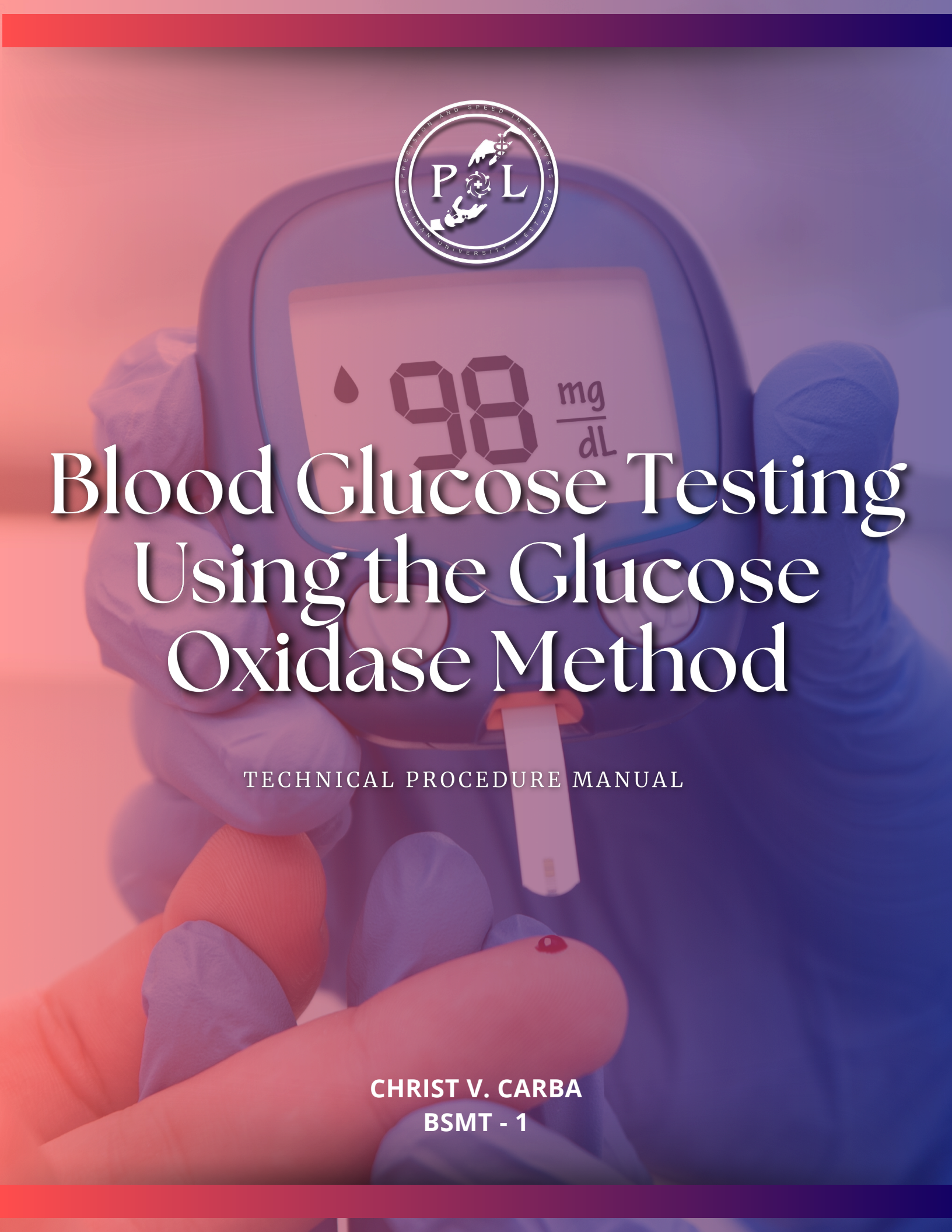

Blood Glucose Testing Using the Glucose Oxidase Method

TECHNICAL PROCEDURE MANUAL

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BSMT - 1

Principle

Glucose is the primary energy source for the body, obtained from food or produced during fasting through gluconeogenesis and glycogenolysis. Normal blood glucose levels range from 80 to 120 mg/dL.

The glucose oxidase method is used to measure blood glucose levels. In this method, glucose oxidase catalyzes the oxidation of glucose in the presence of oxygen, producing gluconic acid and hydrogen peroxide. The hydrogen peroxide then oxidizes a chromogen, causing a color change, or it can be measured with an oxygen-specific electrode. This method specifically measures β-D-glucose, making it widely used in clinical and home settings for its specificity, affordability, and reliability.

Specimen requirements

Type of Specimen:

- Venous blood or capillary blood.

Collection Method:

- Collect 5 mL of venous blood using a fluoride oxalate tube to prevent glycolysis and maintain glucose stability. Capillary blood can also be collected using a lancet for fingerstick testing.

Labeling Requirements:

- Ensure the specimen is labeled with the patient's ID, date, and time of collection.
- Store the specimen at 4°C if testing is delayed and process the sample within 4 hours for accurate results.

Transport Conditions:

- Transport the specimen at 4°C and ensure it reaches the lab within 4 hours.

Rejection Criteria:

- Specimens are rejected if they are hemolyzed, clotted, mislabeled, or stored at room temperature for over 6 hours without refrigeration.

Reagents, Media, supplies, and equipment

Reagents:

- Glucose Oxidase Reagent : Prepare a 10% solution of glucose oxidase. Store this solution at 4°C and utilize it within 7 days of preparation.
- Chromogenic Substrate: To aid in the reaction's color development, use a suitable chromogenic material, such as 4-aminoantipyrine. Follow the preparation and storage instructions provided by the manufacturer.

Supplies:

- Fluoride Oxalate Tubes: Used to collect blood to stop glycolysis.
- Cuvettes: Used to store samples while measuring absorbance.
- Pipettes and Tips: For precise liquid measuring.
- Lancets: For drawing blood with a fingerstick (if doing capillary testing).

Equipment:

- Spectrophotometer
- Water Bath
- Refrigerator
- Glucose Standard

Calibration

- Frequency: The spectrophotometer should be calibrated at the start of each day and after every 50 tests.

- Calibration Procedure:

1. Prepare Calibration Standards: Use a glucose standard solution (e.g., 100 mg/dL) and distilled water as a blank for reference.
2. Zero the Spectrophotometer: Place the cuvette with distilled water into the spectrophotometer and set it to zero absorbance.
3. Calibrate with Standard: Insert the cuvette with the glucose standard into the spectrophotometer and note the absorbance reading.
4. Verify Calibration: Perform additional calibrations using different glucose standards to confirm accuracy throughout the measurement range.
5. Document Calibration Results: Log all calibration results in the laboratory logbook, including the date, time, and any corrective actions taken if necessary.

Quality Control

- Control Materials: Use high (about 180 mg/dL) and low (around 70 mg/dL) glucose solutions to check test accuracy.
- Preparation and Storage: Follow the manufacturer's instructions to prepare controls. Store them at -20°C, thaw before use, and throw away any that have been frozen and warmed up more than five times.
- Frequency of Testing: Test quality control at the start of each day, after major equipment maintenance, and after every 10 patient samples.
- Expected Results: High control should be within ±10% of 180 mg/dL, and low control should be within ±10% of 70 mg/dL.
- Corrective Actions: If results are not correct, check for errors, recalibrate the machine, and retest. Write down all actions in the lab logbook.
- Recording: Keep a record of quality control results, including the date, time, values, and any actions taken.

Step by step instructions

Quantitative Testing

1. Collect 5 mL of venous blood in a fluoride oxalate tube or obtain a capillary blood sample through a fingerstick.
2. Using a pipette, transfer 100 µL of the blood sample into a clean cuvette.
3. Add 1 mL of glucose oxidase reagent to the cuvette.
4. Place the cuvette in a water bath at 37°C for 15 minutes to allow the reaction to take place.
5. After incubation, measure the absorbance at 540 nm using a spectrophotometer.
6. Use the absorbance reading to calculate glucose concentration based on the calibration curve. Record the results.

Qualitative Testing

1. Color Change Observation:
    - After incubation, check the cuvette for any color change. A color change indicates the presence of glucose, while no color change suggests its absence.

Interpretation

1. Quantitative Results:
    - Compare the glucose concentration with standard reference ranges. Normal fasting levels generally fall between 70-100 mg/dL.
2. Qualitative Results:
    - Report as "Positive" if there is a color change, indicating glucose presence. If no color change occurs, report the test as "Negative."
3. Abnormal Results:
    - Report any abnormal glucose concentrations (greater than 126 mg/dL for fasting) to the physician for further action.

Reporting Results

Reference Intervals

- Normal fasting glucose levels are 70-100 mg/dL.
- Postprandial glucose levels should ideally be less than 140 mg/dL.

Procedures for Reporting Abnormal Results

- Identify and address abnormal results quickly.
- Report any fasting glucose greater than 126 mg/dL or postprandial glucose exceeding 200 mg/dL to the physician.
- Critical values, defined as less than 20% or greater than 60%, must be reported urgently.
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Procedure Notes

Special Precautions

- Ensure reagents are at room temperature and handle samples as potentially infectious.

Possible Sources of Error

- *Hemolysis*: Can lead to falsely elevated glucose levels.
- *Calibration Issues*: Ensure the spectrophotometer is calibrated correctly.
- *Contamination*: Use fresh pipette tips to prevent cross-contamination.

Common Problems

- If the spectrophotometer shows an error, check the power source and calibration.
- Inconsistent results may indicate calibration or reagent issues.

Limitations of Methods

1. False elevation of glucose levels can occur due to hemolysis
2. Substances such as ascorbic acid may interfere with the results

3. The accuracy of the method depends on the proper calibration of the spectrophotometer
4. This method specifically measures β-D-glucose, potentially missing other sugars
5. Delayed testing can lead to degradation of glucose levels

Troubleshooting

1. If the spectrophotometer malfunctions, check the power supply and recalibrate the device before retesting.
2. If quality control values are out of range, review all procedures and repeat the test using fresh controls.
3. In case of equipment failure, utilize a second validated centrifuge or send samples to a reference laboratory for testing.
4. If results are inconsistent, recheck sample collection and processing methods for errors.

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Effective Date

The effective date of this procedure is _____.

Signature of Laboratory Director

CHRIST V. CARBA
[Laboratory Director's Name]