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Liquid Glucose (Oxidase) Reagent Set

Intended Use
For the quantitative determination of Glucose in serum. For *in vitro* diagnostic use only.

Clinical Significance
The determination of glucose in serum is most commonly performed for the diagnosis and treatment of diabetes mellitus.

Test Summary
Early enzymatic methods for glucose determination used Glucose Oxidase to catalyze the oxidation of glucose to hydrogen peroxide and gluconic acid.[1] The hydrogen peroxide that is formed is measured by the oxidation of a chromagen.[2] Many chromagens were investigated but many were discarded because of possible carcinogenicity, toxicity, instability or because they were affected by many interfering substances. Trinder[3] modified Emerson[4] to develop an efficient peroxidase-phenol-aminophenazone system for the quantitation of hydrogen peroxide by formulation of a red quinoneimine dye. This method is less influenced by interfering substances and does not suffer from the many drawbacks of earlier methods.

Principle
Glucose Oxidase

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-Gluconate}$$

POD

$$H_2O_2 + \text{4-AAP} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + H_2O$$

Glucose is oxidized by glucose oxidase to gluconate and hydrogen peroxide. Phenol + 4-AAP + hydrogen peroxide, in the presence of peroxidase, produces a quinoneimine dye that is measured at 500nm. The absorbance at 500nm is proportional to the concentration of glucose in the sample.

Reagent Composition
Glucose Oxidase (microbial) 12,000 u/l, Peroxidase (horseradish) > 1,000 u/l, 4-AAP >0.3mM, Phenol 4mM, Buffer, pH 7.4 ± 0.1, non-reactive stabilizers, preservative. See "Precautions".

Reagent Preparation
The reagent is ready to use.

Reagent Storage and Stability
1. The reagent should be stored refrigerated at 2-8°C.
2. The reagent is stable until the indicated expiration date when stored as directed.

Precautions
1. Reagent is intended for *in vitro* diagnostic use only.
2. The reagent should not be used if it has developed turbidity or other evidence of microbial growth.
3. The reagent should not be used if it fails to meet linearity claims or fails to recover control values in the stated range.
4. All specimens and controls should be handled as potentially infectious, using safe laboratory procedures. (NCCLS M29-T2).[5]

Specimen Collection and Storage
1. Non-hemolyzed serum or heparinized plasma is recommended
2. Serum must be separated from the clot promptly since the rate of glucose decrease is approximately 7% per hour in whole blood.[6]
3. Glucose in serum is stable for twenty-four hours when stored refrigerated (2-8°C).
4. Specimens should be collected as per NCCLS document H4-A3.[7]

Interferences
1. Grossly lipemic samples may cause falsely elevated glucose values.
2. Bilirubin to the level of 20 mg/dl and Hemoglobin to a level of 500 mg/dl have both been found to exhibit negligible interference (<3%) in this assay. NOTE: Glucose level was 184 mg/dl for the Bilirubin study and 188 mg/dl for the Hemoglobin study.
3. Young, et al[8] has published a comprehensive list of interfering substances.

Materials Provided
Glucose reagent.

Materials Required but not Provided
1. Accurate pipetting devices (1.0 ml & 10ul)
2. Test tubes
3. Timer (To measure ten minutes)
4. Spectrophotometer able to read at 500 nm
5. Heating block (37°C)
6. Serum controls with known normal and abnormal glucose values

Procedure (Automated-General)

Wavelength:	500 nm
Assay Type:	Endpoint
Sample/Reagent Ratio:	1:101
Reaction Direction:	Increasing
Temperature:	37˚C
Incubation Time:	600 seconds
Low Normal:	70 mg/dl
High Normal:	105 mg/dl

Procedure (Manual)
1. Label test tubes labeled "blank", "control", "standard", "patient", etc.
2. Pipette 1.0ml of working reagent to all tubes and place in a 37°C heating bath for at least five minutes.
3. Add 0.01ml (10 ul) of sample to respective tubes. Mix and incubate at 37°C for ten minutes.
4. After incubation, zero spectrophotometer with the reagent blank. Read and record the absorbances of all tubes at 500nm (500-520nm).
5. To determine results see "Calculations" section.

Limitations
1. The reagent gives linear results over the range of 0-500 mg/dl. Samples that exceed 500 mg/dl should be diluted with an equal volume of saline and re-assayed. Multiply result by two.
2. If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.03ml (30ul) of sample to 3.0ml of reagent. Perform the test as described above.

3. Lipemic sample may give falsely elevated results. To correct for lipemia a serum blank must be run. Serum Blank: Add 0.01ml (10ul) of sample to 1.0ml water. Zero spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance. Calculate as usual.

Calibration

Use a NIST-traceable glucose standard (100mg/dl) or serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Calculations

Abs. = Absorbance

$$\frac{\text{Abs. (Patient)}}{\text{Abs. (Standard)}} \times \text{Concentration of Standard (mg/dl)} = \text{Glucose (mg/dl)}$$

Example:
Abs. (Patient) = 0.300
Abs. (Standard) = 0.200
Concentration of Std. = 100 mg/dl

Then: $\frac{0.300}{0.200} \times 100 = 150$ mg/dl

SI Units

To obtain results in SI units (mmol/L), multiply the results in mg/dl by ten to convert dl to liter and divide the value by 180, the molecular weight of glucose.

$$\text{mg/dl} \times \frac{10}{180} = \text{mg/dl} \times 0.0556$$

Example: 150mg/dl x 0.0556 = 8.34 mmol/L

Quality Control

Serum controls with known normal and abnormal glucose values should be run routinely to monitor the validity of the reaction. These controls should be run at least with every working shift in which glucose assays are performed. Control values should fall within the established ranges for the particular controls that are in use. It is recommended that each laboratory establish their own frequency of control determination.

Expected Values [9]

70-105mg/dl
It is strongly recommended that each laboratory establish its own normal range.

Performance

1. Assay Range: 0 - 500 mg/dl
2. Correlation: Results obtained with this reagent (y) in 132 samples, ranging in glucose concentration from 32-297 mg/dl, were compared with this obtained in the same samples using a dry-powder reagent (x) based on the same methodology on an automated analyzer. The
3. correlation coefficient was 0.999 and the regression equation was y=1.02x – 1.13. (Sy-x=15.43)
4. Precision: Precision studies were performed on an automated analyzer following a modification of the guidelines contained in NCCLS document EP5-T2.[10]

Within Run			Day To Day		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
101	1.1	1.1	86	2.1	2.5
172	1.3	0.7	198	6.3	3.2
293	3.9	1.3	283	9.2	3.3

5. Sensitivity: The sensitivity for the Liquid Glucose (Oxidase) reagent was investigated by reading the change in absorbance at 500nm for a saline sample, and a serum with a known concentration. Ten replicates of each sample were performed. The results of this investigation indicated that, on the analyzer used, the Liquid Glucose (Oxidase) reagent showed little or no reagent drift on a zero sample. Under the reaction conditions described, 1 mg/dl of glucose gives an absorbance of 0.002.

References

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