POINTE SCIENTIFIC, INC.
5449 Research Drive • Canton MI 48188 • USA

# Glucose Oxidase Reagent Set

## Intended Use
For the quantitative determination of glucose in serum.

## Method History
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. The present procedure is based on the above principle but utilizes a non-corrosive phenol substitute for added safety and convenience.

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

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{Hydroxybenzoate} \xrightarrow{\text{POD}} \text{Quinoneimine dye} + H_2O$$

## Reagents
(Concentrations refer to reconstituted reagent)
Glucose Oxidase >15u/ml, Peroxidase (horseradish) 1.2u/ml, 4-Aminoantipyrine 0.38mM, Phosphate Buffer, pH 7.5±0.1, Sodium p-Hydroxybenzoate 10mM, non-reactive Stabilizers and fillers, Sodium Azide 0.1%.

## Reagent Preparation
Empty contents of one vial into the volume of distilled water stated on the vial label. Swirl to dissolve. Store reconstituted reagent in an AMBER bottle at 2-8°C.

## Reagent Storage
1. Dry reagent and standard should be stored refrigerated at 2-8°C and is stable until the expiration date.
2. Reconstituted reagent should be stored in an AMBER container and is stable for 30 days when stored at 2-8°C.

## Reagent Deterioration
Do not use if:
1. The dry reagent has been exposed to moisture and caking has occurred.
2. The reagent fails to meet linearity claims or fails to recover control values in the stated range.

## Precautions
This reagent is for *in vitro* diagnostic use only.
This reagent contains sodium azide at 0.1%. This may react with copper or lead plumbing to form explosive metal azides. Upon disposal, flush with large amounts of water to prevent azide build up.

## 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.[5]
3. Glucose in serum or plasma is stable for 24 hours when stored refrigerated at 2-8°C.

## Interferences
1. Grossly lipemic or icteric samples will cause false glucose values, consequently a patient blank should be run. Add 0.01ml (10ul) of patient sera to 1.0ml distilled water and read against a water blank. Subtract this absorbance from the patient test absorbance to correct for the lipemia or icterus.
2. Young, et al[11] has published a comprehensive list of drugs and substances that may affect glucose values.

## Materials Provided
Glucose reagent.

## Materials Required but not Provided
1. Accurate pipetting devices
2. Test tubes/rack
3. Timer
4. Spectrophotometer able to read at 500 nm.
5. Graduated cylinder
6. Heating block (37°C)

## Procedure (Automated)
Refer to specific instrument application instructions.

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

## Procedure Notes
1. Final color is stable for at least fifteen minutes.
2. If the spectrophotometer being used requires a final volume greater than 1.0ml for accurate reading, use 0.02ml (20ul) of sample to 3.0ml of reagent. Perform the test as described above.

## Limitations
Concentrations exceeding 500 mg/dl should be diluted with saline 1:1, re-run and the final answer multiplied by two.

## Calibration
Use an aqueous Glucose Standard (100mg/dl) or an appropriate serum calibrator.

## Calculations

Abs. = Absorbance

$\frac{\text{A (Patient)}}{\text{A (Standard)}}$ x Concentration of = Glucose (mg/dl)
Standard (mg/dl)

## SI Units

To obtain results in SI units (mmol/L), multiply your 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

Use of normal and elevated control sera of known glucose concentrations are recommended to test the validity of the reaction.

## Expected Values [7]

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

## Performance

1. Linearity: 500 mg/dl
2. Comparison: A study performed against another commercial glucose reagent using a similar methodology yielded the linear regression equation y=0.98x+2.0 with a correlation coefficient of 0.998.
3. Precision:

| Within Run | | | Run To Run | | |
|---|---|---|---|---|---|
| **Mean** | **S.D.** | **C.V.%** | **Mean** | **S.D.** | **C.V.%** |
| 97 | 0.6 | 0.6 | 96 | 1.8 | 1.9 |
| 221 | 1.3 | 0.6 | 217 | 3.1 | 1.4 |

## References

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Manufactured by Pointe Scientific, Inc.
5449 Research Drive Canton, MI 48188

"European Authorized Representative"
(O.E.A.R.C.) Av. De Tervueren 34 bte
44 B-1040 Brussels, Belgium

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