Glucose and Diabetes

The purpose of this technical bulletin is to provide information about diabetes, applications for glucose testing and proper glucose testing on the Cholestech LDX®.

Diabetes Mellitus

Diabetes mellitus is a complex disorder of carbohydrate, fat and protein metabolism. It is primarily a result of a defect in the secretion or action of insulin, the hormone that facilitates and controls the use of glucose in the cells. Because of the deficiency of insulin, diabetic patients have an impaired tolerance to glucose, that leads to a number of short term and long term complications.

The long term complications of diabetes mostly involve vascular problems. Microvascular problems include peripheral neuropathy (nerve damage), nephropathy (kidney disease) and retinopathy (retinal damage, causing decreased vision and blindness). The macrovascular manifestations of diabetes involve accelerated arteriosclerosis, that result in both ischemic heart disease and peripheral vascular disease.

The short term dangers of diabetes mellitus can be life threatening. They are related to a difficulty controlling insulin and glucose levels, and primarily occur in insulin dependent diabetic patients. When there is insufficient insulin, which is needed for glucose metabolism, the body burns fat for energy. This results in ketoacidosis (accumulation of ketones and acid in the blood) that can lead to diabetic coma. Hypoglycemia (low blood sugar) is common in diabetic patients receiving insulin, because of difficulties in adjusting their insulin levels. Unless treated promptly by administering glucose, this hypoglycemia can result in coma and death.

The Different Types of Diabetes

Type 1 (previously referred to as Insulin Dependent Diabetes Mellitus) comprises about 10 percent of all cases. Diagnosis of Type 1 Diabetes usually occurs when the patient is between 10 and 30 years, but the disease can occur at any age. Type 1 Diabetes is believed to be caused by an autoimmune response (by the person’s own immune system) that results in the destruction of the insulin producing cells of the pancreas. As a result, Type 1 diabetic patients are dependent on treatment with insulin injections to control glucose and other energy metabolism. Because insulin is a potent hormone, Type 1 patients often experience wide and unstable fluctuations in blood glucose concentrations. Control of Type 1 Diabetes is facilitated by frequent monitoring of blood glucose levels.

Type 2 (previously referred to as Non-Insulin Dependent Diabetes Mellitus) comprises about 90 per cent of all cases. Type 2 Diabetes is thought to involve several distinct or combined defects that result in either insulin deficiency, where there is an insufficient amount of insulin to maintain “normal” glucose physiology, or insulin resistance, where the response to insulin by the cells of the body is blunted. Sixty to ninety percent of Type 2 diabetic patients are obese; weight loss generally improves the condition.

Secondary Diabetes Mellitus: Other diseases, such as pituitary adenoma, acromegaly, pancreatitis, and hemochromatosis can result in secondary diabetes mellitus. The features of secondary diabetes mellitus usually resolve with successful treatment of the primary condition.

The Public Health Impact

Including known and undiagnosed disease, diabetes mellitus is estimated to affect 6.6% of the population. Assuming a population of 250 million, this means there are approximately 16.5 million people with diabetes in...
the US. Approximately 1.6 million of these have Type 1 Diabetes, while almost 15 million have Type 2 Diabetes. Only half of those with Type 2 Diabetes have been identified. Screening for these patients who have non-symptomatic diabetes is important, because proper treatment will minimize the long term complications of the disease. The incidence and prevalence of diabetes mellitus vary with age, affecting about 2% of those under 40, increasing steadily to more than 17.5% in those older than 60 years.

According to the new ADA recommendations, all adults >45 years old should have a fasting plasma glucose measurement every 3 years unless they are already diagnosed with diabetes.

**Diagnosis**

The demonstration of significant hyperglycemia (high blood glucose) is the key to the diagnosis of diabetes mellitus. For Type 1 Diabetes, the diagnosis is usually simple, since hyperglycemia appears abruptly, is severe, and is accompanied by serious metabolic derangements (e.g., metabolic ketoacidosis). It is in Type 2 Diabetes that early diagnosis becomes troublesome. The risk for the later development of microvascular disease makes it important to identify patients with Type 2 Diabetes early.

The American Diabetes Association (ADA) has recently (July 1997) revised its criteria for clinical diagnosis of diabetes. Three ways to diagnose diabetes are possible:

1) Symptoms of diabetes plus casual plasma glucose concentration \(>200 \text{ mg/dL (11.1 mmol/L)}\). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include excessive passage of urine, excessive or abnormal thirst and unexplained weight loss.

2) Fasting plasma glucose \(>126 \text{ mg/dL (7.0 mmol/L)}\). Fasting is defined as no caloric intake for at least 8 hours.

3) 2 hour postload glucose \(>200 \text{ mg/dL during an oral glucose tolerance test.}\)

Any of the above abnormal glucose levels must be confirmed, on a subsequent day, by any one of the three methods listed above. When screening for diabetes, any abnormal glucose result should be referred to a physician for further follow up.

**Hypoglycemia**

Hypoglycemia (low blood glucose) as a laboratory diagnosis has no specific defined limits. It is not uncommon for an isolated plasma glucose concentration to be as low as 50 mg/dL several hours after the ingestion of oral glucose; even in the fasting state, there may be an occasional extremely low blood glucose value without concurrent symptoms or evidence of underlying disease. An accurate interpretation of a single low blood glucose value is impossible without knowledge of the clinical setting in which it occurs.

There are no symptoms that are specific for hypoglycemia. A rapid fall in plasma glucose will usually trigger the release of epinephrine, and it is epinephrine that accounts for the signs and symptoms which are most commonly attributed to “hypoglycemia” (i.e., weakness, shakiness, sweating, nausea, rapid pulse, light-headedness, hunger, and epigastric discomfort). The symptoms may occasionally be triggered by a rapid drop in blood glucose concentration, even though the blood glucose may not fall below the reference range. These symptoms are termed *adrenergic* and may occur 2-3 hours after eating. A very low level of plasma glucose (<20-30 mg/dL (<1.1-1.7 mmol/L)) causes impairment of central nervous system function, and results in confusion, lethargy, seizures or loss of consciousness. These symptoms are known collectively as *neuoglycopenia*.

When planning a hypoglycemia evaluation, it is helpful to consider whether hypoglycemia occurs spontaneously in the fasting state or seems to be triggered by the ingestion of food, because appropriate diagnostic strategies for these two situations are considerably different.

**Fasting Hypoglycemia**

Fasting hypoglycemia in adults is rare. A precise point for separation between the low range of normal and abnormally low is not possible; values as low as 30 mg/dL may be seen in healthy premenopausal women after a 72-hour fast. However, fasting plasma glucose concentrations <50-60 mg/dL (2.8-3.3 mmol/L) are uncommon and should be investigated.

**Postprandial Hypoglycemia**

In most cases, the question of hypoglycemia will be raised when a patient complains of *adrenergic* symptoms that occur ~2 hour after eating and seem to be relieved by food intake. There are specific situations in which these symptoms are likely to be related to low blood glucose; for example, in early diabetes mellitus, when insulin release is delayed and exaggerated, or after gastrointestinal surgery with rapid gastric emptying. However, many individuals with adrenergic complaints in the postprandial setting exhibit these symptoms when their blood glucose is clearly normal. The best diagnostic strategy is to obtain a blood specimen at the time the patient is experiencing the symptoms. Finding a normal blood glucose at a time when symptoms are present is strong evidence that the symptoms are not related to hypoglycemia. A blood glucose concentration <60 mg/dL (< 3.3 mmol/L) with associated adrenergic symptoms would suggest a cause-and-effect relationship.

**Glucose Test Methods**

There are a variety of glucose testing methods. Most of them employ enzymes, that react with glucose and ultimately form a colored product. The glucose concentration correlates with the amount of color formed. The
Cholestech LDX glucose test uses a glucose oxidase enzyme that catalyzes the oxidation of glucose to gluconolactone and hydrogen peroxide. Reaction of the hydrogen peroxide in the presence of the enzyme peroxidase and a chromogenic (color forming) oxygen acceptor, such as TOOS, results in the formation of a color that can be measured (see Chart A).

Glucose oxidase is highly specified and will only react with D-glucose. The second step, involving peroxidase, is much less specific than the glucose oxidase reaction. Various substances such as uric acid, ascorbic acid, bilirubin and glutathione can inhibit this reaction, resulting in lower values. Consult your test cassette Product Inserts for information on these and other interferences.

The YSI 23A (Yellow Springs Instrument Co., Yellow Springs OH) is frequently used as a reference method for whole blood glucose testing. It is one of the reference methods at Cholestech. The YSI 23A uses glucose oxidase but the methodology is significantly different from other tests. The glucose oxidase is immobilized on a thin layer of membrane. When a blood sample is introduced, glucose diffuses through the membrane and reacts with the enzyme to produce hydrogen peroxide. The latter then contacts a platinum anode, where oxidation takes place. This produces an electrical current, which is directly proportional to the glucose concentration in the sample.

Collecting and Handling of Specimens

Capillary whole blood samples should be tested on the Cholestech LDX within five minutes of collection. Plasma or serum samples should be separated from the blood cells within 30 minutes after blood is drawn if accurate glucose values are to be obtained. When blood is drawn and permitted to clot and stand uncentrifuged at room temperature, the average decrease in glucose is 7-10 mg/dL per hour. This decrease is the result of glycolysis (metabolism of glucose by the red blood cells). Plasma removed from cells after moderate centrifugation still contains white blood cells that also metabolize glucose. In separated, unhemolyzed serum, the glucose concentration is generally stable up to eight hours at room temperature if kept free of bacterial contamination, and up to 72 hours when stored at 4°C.

Glucose levels measured on the Cholestech LDX may show significant differences from a capillary sample drawn at the same time and tested on a hand held glucose meter. On a hand held glucose meter the glucose is measured in whole blood. The red blood cells have a solid phase which does not contain glucose and thus has a dilution effect on the glucose concentration causing lower glucose results. Plasma glucose levels, measured on the Cholestech LDX, will be approximately 12% higher than whole blood glucose levels measured on a hand held glucose meter.

Blood glucose concentration may vary depending on the source of the sample: arterial, capillary or venous. Arterial blood has the highest glucose levels, followed by capillary blood, then venous blood. In the fasting state, arterial glucose levels are approximately 5 mg/dL higher than capillary levels. Capillary glucose levels are 2-5 mg/dL higher than venous levels. After eating, glucose levels in arterial and capillary blood may be 20-70 mg/dL higher than levels in venous blood. These differences must be taken into account when comparing nonfasting capillary glucose results on the Cholestech LDX with results from serum drawn at the same time and measured on a reference method.

Normal Values

The following chart lists normal values for plasma/serum glucose.

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* Patient consumes a breakfast, lunch, or a glucose solution (75g) and remains at rest during the following 2 hours.

Chart A

\[ \text{b-D-Glucose} + \text{O}_2 \xrightarrow{\text{glucose oxidase}} \text{O-D-Gluconolactone} + \text{H}_2\text{O}_2 \]

\[ 2\text{H}_2\text{O}_2 + 4\text{AAP} + \text{TOOS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine dye} + \text{H}_2\text{O} \]

To assist you with any further questions, please call Technical Service:

800-733-0404

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