



NEAR EAST UNIVERSITY

Faculty of Engineering

Department of Biomedical Engineering

B.S Project

COMPARATIVE STUDY OF THEORY AND  
FUNDAMENTALS OF NON-INVASIVE MEASUREMENTS  
OF BIOCHEMICALS IN THE BLOOD  
WITH AN EMPHASIS ON BLOOD GLUCOSE

by

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## ABSTRACT

In this project, the vital blood minerals and biochemical are discussed along with their normal blood levels. For the accurate measurement of these levels, the three major groups of measurement techniques are introduced: The invasive, the minimally invasive and the non-invasive measurement techniques. Due to the drawbacks of invasive and minimally invasive measurements like risk of contamination from blood drawing and irrepeatability factors, the shift of these measurements to non-invasive methods is necessary. The currently available methods for non-invasive measurements as well as their limitations are thoroughly discussed. Then the devices, based on non-invasive methods, are discussed under four categories: The ones approved and currently available on the market, the ones approved but then pulled back from the market, the ones waiting approval and the ones with not enough available data. The limitations as well as correlation values of these devices with currently standardized measurement devices are discussed. In summary, I suggest that since each measurement device has its innate limitations based on the principle of measurement technique, what should be prioritized is the miniaturization of the available techniques in order to integrate them together in one device so that each methods can make-up for the drawbacks of the other resulting in a device with as accurate measurements as the standardized invasive devices.

## **ACKNOWLEDGEMENTS**

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## 1. Introduction

Blood, which is composed of RBCs, WBCs and various other elements is the essential fluid for bodily functions. Amongst the other elements in blood, the four vital minerals are sodium, potassium. Total serum iron and total calcium and exists the six vital biochemical, glucose, urea, creatinine, bilirubin, ALT and AST. Because these biochemicals are vital, we need to be able to continuously monitor their blood levels and regulate them if necessary and in case, the body cannot achieve this functionality by homeostasis due to some reason.

Perhaps the most important biochemical out of all is glucose, due to the high population of individuals world-wide with the inability to regulate it. Therefore, currently, majority of the resources are being directed towards research in methods for non-invasive glucose monitoring and devices for the application of these methods.

The first discovered method, and currently most accurate still is the invasive methods. Then minimally invasive methods were developed, which are also pretty accurate. Now, the topic of research essentially shifted to non-invasive methods due to various advantages in continuous monitoring and etc.

The non-invasive methods involve various techniques categorizes as either optical or not. The non-optical ones are called the other techniques in this context. The optical techniques discussed in this paper are, Mid-infrared Spectroscopy, Fluorescence, Time of Flight, Near infrared Spectroscopy, Raman Spectroscopy, Photoacoustic Techniques, Scattering Changes (Spatially Resolved Diffuse Reflectance and Optical Coherence Tomography), Polarization Changes, Temperature Modulated |Localized Reflectance, Frequency Domain Reflectance Technique, Ocular Spectroscopy and Fluid Harvesting. The other techniques are Photonic Crystal Glucose Sensing, Metabolic Heat Confirmation Method (Thermal Spectroscopy), Impedance Spectroscopy, Electromagnetic Sensing and Iontophoresis.

In the context of this paper, we will also be discussing the various devices employing invasive, minimally invasive and non-invasive methods produced until today and then compare and contrast the most recent ones employing non-invasive techniques.

## 2. Background

### 2.1 History of Blood Analysis and Devices used

The invention of optical microscope was the first breakthrough of science in order to investigate biological components in more detail visually. See Figure 1 for some of the earlier optical microscopes used in the 18th century currently residing in museum.



Figure 1: *18th century microscopes from the Musée des Arts et Métiers, Paris* <sup>[1]</sup>

This invention was truly the first milestone of micro-science fields such that it enabled us to see objects, organisms and details we were unable to observe with naked eye until then. The first red blood cells were identified in late 1600s using early microscopes.

In early 1900s, the discovery of main blood groups, A, B, AB and 0 yielded higher success with blood transfusion and became a milestone in understanding the reason behind the failure of blood transfusions carried out prior to this discovery.

Centrifuge, although has been around since 1700s, has been used earlier as a milk separator. The application of sedimentation principle to the separation of the blood components like blood plasma from heavier cells like erythrocytes was not until its discovery by Dr Charles R. Drew in late 1930s. This led to longer lasting blood storage for transfusions and decreased contamination.

“The Coulter Principle relies on the fact that particles moving in an electric field cause measurable disturbances in that field. The magnitudes of these disturbances are proportional to the size of the particles in the field.”[4] In this principle, the particles need to be suspended in a conductive liquid medium. The physical constriction of electric field so that current changes due to particle movement can be detected. The particles need to be diluted to ensure the passing through of the particles through the constriction one by one in order to eliminate coincidence factor. The coulter principle can be used in characterization of human blood cells. Wallace H

Coulter invented this method in the late 1940s. The device that employs this principle is called a coulter counter, which became a building block of the first cell sorter and development of flow cytometry.

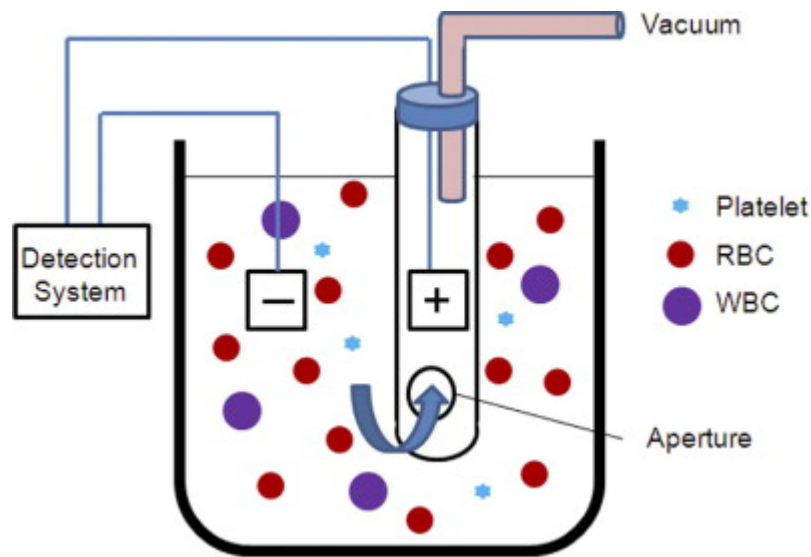


Figure 2: Image demonstrating the coulter principle<sup>[5]</sup>

“X-ray crystallography is a method of determining the atomic and molecular structure of a crystal, in which the crystalline atoms cause a beam of X-rays to diffract into many specific directions.”[6] The structure of haemoglobin, the main protein in erythrocyte that transports oxygen, was unravelled using this method in 1959 by Dr Max Preutz.

Full blood counting is a technique that gives the total number of each type of cell in a person’s blood and was first used by Alexander Vastem in early 1960s. In earlier days, this FBC technique was carried out manually such that a sample of blood is first diluted with a certain dilution factor to a specific volume in the counting chamber. Then number of each kind of cell in the chamber is counted by eye. Then these values are used to obtain a percentage of a certain type of cell in the blood per litre. Nowadays, the FBC is carried out by an automated cell counter, in which the blood sample is forced through a thin tube, while sensors determine the type of each cell by size or characteristics and count the number of each type.

Robot Chemist was introduced in 1966 for the automation of the common wet lab procedures. Although, the initial models were primitive, they became building blocks for today’s most widely used fully automated systems.

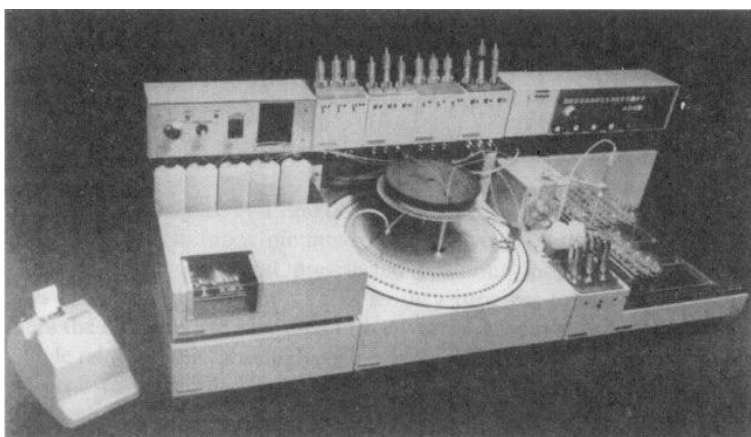


Figure 3: *An earlier robot chemist*<sup>[9]</sup>

## 2.2 Components of Blood today

Blood makes up about 7-8% of the homo sapiens total body weight. This essential fluid carries out functions, vital to organism's survival, like transporting oxygen from the lungs, the site of gaseous exchange, to the tissue fluid all around the cells of the organism; carbon dioxide from the tissue fluid to the lungs; ammonia and other waste products from the tissue fluid to the appropriate sites of disposal. Moreover, blood also plays a vital role in immune-resistance and homeostasis such that it helps regulate body temperature and keep it relatively constant. Blood is made up of erythrocytes, leukocytes, platelets and plasma. All the insoluble and soluble components are suspended and dissolved in the plasma, respectively.

Erythrocytes, also commonly known as the red blood cells make up of 40 to 50% of the total blood volume. This is equivalent to there being 40 to 6 million erythrocytes per mm<sup>3</sup> of blood. These insoluble cells, suspended in the plasma, lack nuclei, specialize in oxygen transport due to their gas-transporting protein molecules called haemoglobin, which contains the heme group (complex iron containing group) during their life time of 120 days. They are produced continuously in the red bone marrow of skull, ribs and the vertebrae as myeloid stem cells, which specializes into erythroblasts, which produces erythrocytes, and disposed of in the liver or spleen, when they die.

Leukocytes, commonly known as the white blood cells, make up a small percentage of blood, about 1% in healthy individuals. Agranular lymphocytes and monocytes and granular basophils, eosinophils and neutrophils are different types of leukocytes that can be found suspended in the blood plasma. These cells play a vital role in immunoresponse to foreign elements that enter the organism. They act as first response mechanisms to bacteria, viruses and fungi, in most cases by tagging them by binding to identify for removal and then removal by

phagocytosis, if possible once identified. They also dispose of the dead or dying tissue, which may become problematic if left undisposed of.

Platelets, also known as thrombocytes, play a vital role in blood clotting. They carry out this function by adhering to the rupture blood vessel walls clogging the rupture. This is a thoroughly controlled mechanism by 13 different clotting factors since spontaneous clotting would be fatal to the organism. Platelets are also produced at the bone marrow from the myeloid stem cells (same origin as the erythrocytes), which specialize into megakaryoblasts, which produces megakaryocytes, which in return produces thrombocytes.

Plasma makes up of the 55% of the blood volume and is mainly made up of water, sugar, fat, protein and salts. Hormones, vitamins, clotting factors, antibodies, enzymes and various other proteins are also within the plasma whether in soluble or insoluble form. Approximately, 500 types of proteins produced by the human body have been identified in the plasma so far. It is also from this plasma that the biochemical analysis of the other chemical components of the blood can be carried out. A basic metabolic panel shows sodium, potassium, chloride, bicarbonate, blood urea nitrogen (BUN), magnesium, creatinine, glucose and calcium levels in the plasma. Cholesterol, glucose levels as well as the presence and lack of STDs can also be determined from blood tests. As for glucose level tests, regular glucose test is made as a one-time test at a certain examination, whereas glucose tolerance test involves repeated testing with short intervals. Please see Table 1 for the normal ranges of some of the blood plasma components expected during a blood test.



Test	Lower limit	Upper limit	Unit
Sodium (Na)	135, 137	145, 147	mmol/L or mEq/L
	310, 320	330, 340	mg/dl
Potassium (K)	3.5, 3.6	5.0, 5.1	mmol/L or mEq/L
	14	20	mg/dl
Chloride (Cl)	95, 98, 100	105, 106, 110	mmol/L or mEq/L
	340	370	mg/dl
Ionized calcium (Ca)	1.03, 1.10	1.23, 1.30	mmol/L
	4.1, 4.4	4.9, 5.2	mg/dL
Total calcium (Ca)	2.1, 2.2	2.5, 2.6, 2.8	mmol/L
	8.4, 8.5	10.2, 10.5	mg/dL
Total serum iron (TSI) - male	65, 76	176, 198	µg/dL
	11.6, 13.6	30, 32, 35	µmol/L
Total serum iron (TSI) - female	26, 50	170	µg/dL
	4.6, 8.9	30.4	µmol/L
Total serum iron (TSI) - newborns	100	250	µg/dL
	18	45	µmol/L
Total serum iron (TSI) - children	50	120	µg/dL
	9	21	µmol/L
Total iron-binding capacity (TIBC)	240, 262	450, 474	µg/dL
	43, 47	81, 85	µmol/L
Transferrin	190, 194, 204	326, 330, 360	mg/dL
	25	45	µmol/L
Transferrin saturation	20	50	%
Ferritin - Male	12	300	ng/mL
	27	670	pmol/L
Ferritin - Female	12	150	ng/mL
	27	330	pmol/L
Ammonia	10, 20	35, 65	µmol/L
Copper	70	150	µg/dL
	11	24	µmol/L
Ceruloplasmin	15	60	mg/dL
Phosphate ( $\text{HPO}_4^{2-}$ )	0.8	1.5	mmol/L
Inorganic phosphorus (serum)	1.0	1.5	mmol/L
Copper (Cu)	11	24	µmol/L
Zinc (Zn)	9.2, 11	17, 20	µmol/L
Magnesium	0.6, 0.7	0.82, 0.95	mmol/L

**Table 1:** Reference normal ranges for blood tests of minerals <sup>[2]</sup> The normal lowest and highest levels of component expected in a healthy individual are given as above.

## **2.2.1 Vital minerals in blood**

### **2.2.1.1 Sodium(Na)**

Sodium (Na) is an essential nutrient and minimum 500mg sodium need to be taken into the body per day, most commonly by ingestion along with food containing it in the form of table salt, sodium chloride. DRI for sodium is 2.3gr/day and normal levels of sodium are from 135 to 145 mmol/L.

High level of intake promotes hypertension risk. Sodium is lost by sweating or excretion in the urea. The excess of sodium in human diet increases blood pressure. Individuals with high blood pressure are more susceptible to the heart diseases, stroke and kidney damages. High concentrations of salt in blood increases the water content of the body by osmosis and may cause swelling of the legs and hands.

The fluid and sodium amounts within the body are regulated by the rennin-angiotensin system. In response to the decrease in blood pressure and sodium concentration in blood, kidney produces rennin, which then promotes the production of aldosterone by adrenal glands and angiotensin, which reabsorbs sodium from the urine. This increases the sodium concentration in blood, which decreases rennin production and sodium concentration is kept at a regular level by this self-regulating negative feedback mechanism. Other mechanisms involve stimulation of thirst, handling of water and sodium by the kidneys and production of ADH.

Most of sodium in the body is located in the blood and lymph fluid. Firstly, it plays a vital role in neuron functioning. Electrical signals are established and transmitted from one neuron to the next one by means of electrochemical activity involving action potentials, in which sodium is essential to regulate the electrical gradient. Secondly, has a role in osmoregulation between cells and tissue fluid. Concentration gradients determine which direction the solutes and water flow and these concentration gradients are regulated by means of regulating the amount of solutes like sodium dissolved the water. Thirdly, muscle contraction is associated with sodium. For muscle contraction to occur a certain amount of sodium need to enter the muscle cell from the neighbouring neuron through the neuromuscular junction. Last but not least, functioning of Na<sup>+</sup>/K<sup>+</sup>-ATPase, essential trans-membrane protein in action potentials, hence cell electrochemical functioning, depend on sodium.

The sodium concentration of an individual can be measures by drawing blood or from her/his sweat. A patch worn on the skin during exercise absorbs the excreted sweat and this patch can be tested in laboratory by rinsing with pure water and analysing its components. This

is one of the most common ways of non-invasive sodium concentration testing, however, it is time consuming.

Low sodium levels, hyponatremia, are usually associated with low total body water, hypovolemia due to osmotic relationship of the two. Hyponatremia is a dangerous but relatively uncommon disease with symptoms like decreased appetite, nausea and vomiting, difficulty concentrating, confusion, lethargy, agitation, headache.

#### **2.2.1.2 Potassium (K)**

Potassium (K) is another vital electrolyte with cellular and electrical functions in the body. DRI for potassium is 2.5gr/day. Normal levels of potassium are from 3.5 to 5 mmol/L. The erythrocytes contain 420mg of potassium, whereas this amount is 4-5mg in blood serum.

High potassium, low sodium diet is considered ideal and is associated with decrease in blood pressure, hence help prevent hypertension. Such a healthy diet would involve food like fruits and vegetables as well as whole grains.

Potassium absorption from ingested food material takes place in the small intestine. Excess potassium is eliminated from the body mostly in the urine and some in the sweat. This absorption and elimination processes are controlled by the kidneys, keeping blood potassium levels constant. The elimination of potassium is stimulated by the adrenal hormone, aldosterone. Failure to eliminate excess potassium from blood serum may have an adverse effect in action potentials and the highest risk of this is to the heart muscles, which may result in heart attack and heart muscle failures leading to death.

Potassium has important bodily functions. Firstly, together with sodium, it regulates the acid-base balance and water balance of blood and tissues. Secondly, nerve impulse conduction of nerve cells is only possible because of the electric gradients created by potassium and sodium fluxes. The potassium and sodium are usually coupled together in cell-based functions since, in most cases they counteract each other and control of this degree of counteract is what creates action potentials and determines the osmotic gradients. If sodium is the electrolyte responsible for depolarization, then potassium is the element of polarization. Thirdly, muscle contraction and heart beat is also due to electrical potential gradients created by sodium-potassium pumps. Specifically, potassium aids muscles return to their resting states after contraction. Besides these functions coupled with sodium, potassium has functions unique to the electrolyte itself. Potassium is plays a role in protein synthesis from amino acids, a cellular biochemical reaction. Moreover, potassium is also necessary for carbohydrate metabolism. In

this energy metabolism, specifically in glycogen and glucose mechanism, glucose is converted to glycogen in order to store in the liver and potassium plays a role in this mechanism. Last but not least, potassium is also fundamental in muscle building and normal growth. For normal functioning of heart and nervous system, the electrolytic balance of the potassium electrolyte is a must.

The potassium concentration of an individual can be measured in the same ways as for sodium, whether invasive or non-invasive (from urea or sweat). In case of invasive method (lab analysis of drawn blood sample), it is better to determine the total potassium concentration from erythrocytes instead of blood serum, since it is found almost ten times more concentrated in RBC than blood serum.

Hyperkalemia is the condition of elevated potassium levels. However, since excess potassium is eliminated by kidneys, this is an uncommon condition and the most likely underlying cause is renal failure. In case of fully functional kidneys, the excess potassium intake is not a problem as its levels in blood can easily be regulated. Moreover, potassium intake is the prescribed cure for hypertension associated with high sodium intakes. Hypokalemia, on the other hand, is much more commonly occurring disease, which is the deficiency of potassium in blood. This condition is much more serious and failure to cure might result in fatalities. Deficiency of potassium will have adverse effects on normal neural functioning and muscle contractions, hence might result in congestive heart failure and cardiac arrhythmia. Moreover, this also means that the excess sodium intake won't be counteracted by potassium, hence leads to hypertension.

### **2.2.1.3 Total Calcium**

Calcium is the most common mineral in the body since it is also used in bone structure and one of the most important. The serum level of calcium is closely regulated with normal total calcium of 2.2-2.6 mmol/L (9-10.5 mg/dL) and normal ionized calcium of 1.1-1.4 mmol/L (4.5-5.6 mg/dL). Almost all of the calcium in the body is stored in bone. The rest is found in the blood. The amount of calcium in the body depends on the amount of calcium ingested from the food, calcium and vitamin D the intestines absorb, phosphate in the body and certain hormones, including parathyroid hormone, calcitonin, and estrogen in the body.

# Calcium regulation

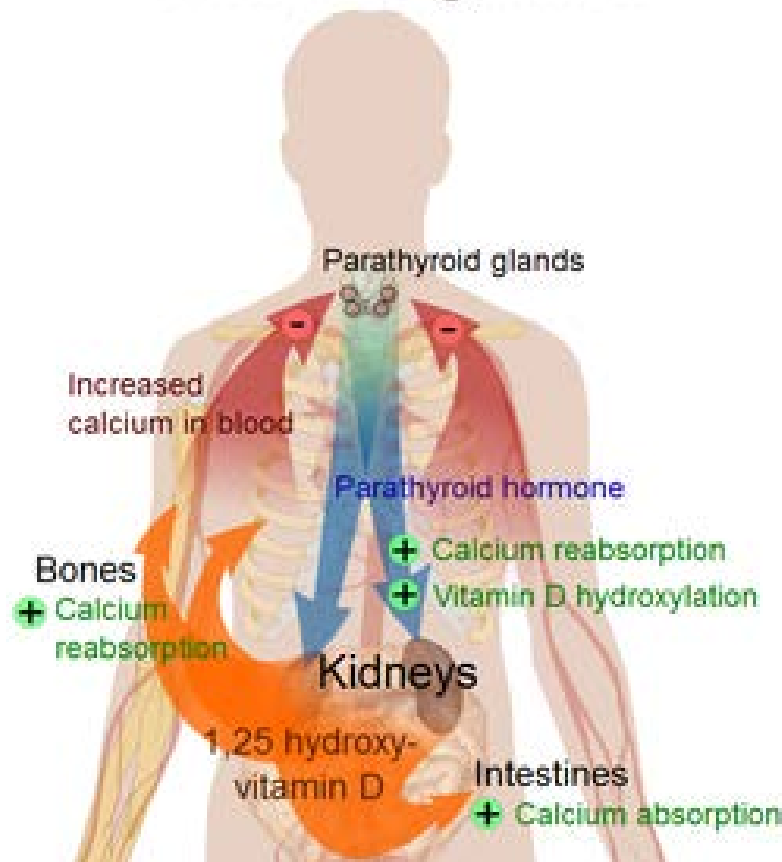


Figure 4: *Calcium regulation mechanism in the body*<sup>[27]</sup>

In the body, calcium is needed to build and fix bones and teeth for structural functioning as supporting material in the form of calcium phosphate. Calcium also helps the nerves function, makes muscles squeeze together, helps blood clot as it acts as a coenzyme for clotting factors, and helps the heart to work.

When blood calcium levels get low (hypocalcemia), the bones release calcium to bring it back to a good blood level. When blood calcium levels get high (hypercalcemia), the extra calcium is stored in the bones or passed out of the body in urine and stool. In ability to maintain a normal calcium level leads to many complications including cardiac arrhythmias.

## 2.2.1.4 Total Serum Iron

Serum iron is the amount of circulating iron that is bound to transferrin. Most of the iron in the body is bound to haemoglobin in RBCs, some to myoglobin molecules and some stored in liver in other forms. The normal range for TSI in males is 65 to 176 µg/dL. Most of the iron in the body is hoarded and recycled by the reticuloendothelial system, which breaks down aged red

blood cells. The majority of the iron absorbed from digested food or supplements is absorbed in the duodenum by enterocytes of the duodenal lining.

When body levels of iron are too low, hepcidin in the duodenal epithelium is decreased. This allows an increase in ferroportin activity, stimulating iron uptake in the digestive system. When there is an iron surplus, more hepcidin is released thus blocking additional ferroportin activity, resulting in less iron uptake. In individual cells, an iron deficiency causes responsive element binding protein to iron responsive elements on mRNA for transferrin receptors, resulting in increased production of transferrin receptors. These receptors increase binding of transferrin to cells, and therefore stimulating iron uptake.

The human body needs iron for oxygen transport. Also, since it can act both as an electron donor and acceptor, iron is necessary in other metabolic reactions. In case of a bacterial infection, the immune system initiates a process known as iron withholding, which is a mechanism that provides the body with bacterial protection.

## 2.2.2 Vital Biochemicals in Blood

### 2.2.2.1 Glucose

Glucose is by far the most common carbohydrate and classified as a monosaccharide, an aldose, a hexose, and is a reducing sugar. It is also known as dextrose, because it is dextrorotatory, which means that as an optical isomer, it rotates plane polarized light to the right and also an origin for the D designation. The normal range for blood glucose is 4.4 to 6.1 [mmol/L](#) (82 to 110 mg/dL), which is measured by a fasting blood glucose test.

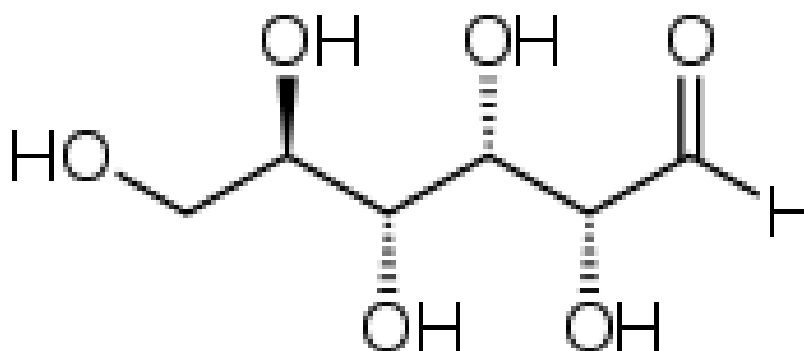


Figure 5: *Glucose as a chain molecule* <sup>[28]</sup>

Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia).

In type 1 diabetes, the body does not produce insulin. Some people may refer to this type as insulin-dependent diabetes, juvenile diabetes, or early-onset diabetes. People usually develop type 1 diabetes before their 40th year, often in early adulthood or teenage years.

In type 2 diabetes, the body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance). Approximately 90% of all cases of diabetes worldwide are of this type.

Gestational diabetes affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose.

Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. This includes the baby being born bigger than normal.

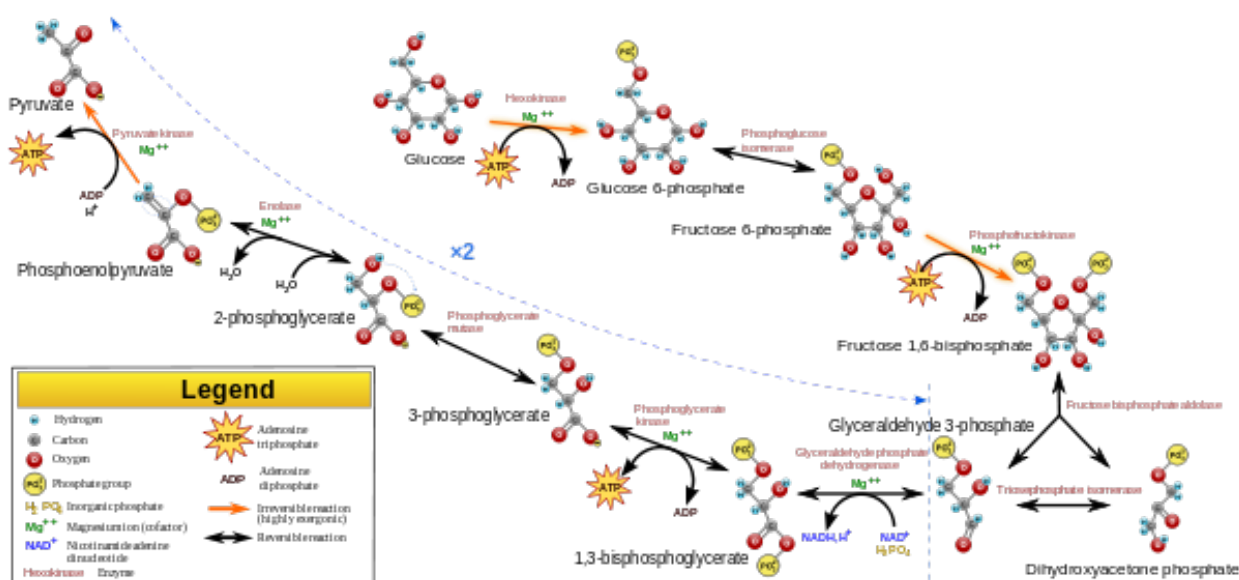
It is due to these conditions that the glucose levels need to be closely regulated in the blood. Please refer to the table 2 below for the list of hormones that play a role in this blood glucose mechanism and regulate the blood glucose levels.

Hormone	Tissue of Origin	Metabolic Effect	Effect on Blood Glucose
Insulin	Pancreatic $\beta$ Cells	1) Enhances entry of glucose into cells; 2) Enhances storage of glucose as glycogen, or conversion to fatty acids; 3) Enhances synthesis of fatty acids and proteins; 4) Suppresses breakdown of proteins into amino acids, of adipose tissue into free fatty acids.	Lowers
Somatostatin	Pancreatic $\delta$ Cells	1) Suppresses glucagon release from $\alpha$ cells (acts locally); 2) Suppresses release of Insulin, Pituitary tropic hormones, gastrin and secretin.	Lowers
Glucagon	Pancreatic $\alpha$ Cells	1) Enhances release of glucose from glycogen; 2) Enhances synthesis of glucose from amino acids or fatty acids.	Raises
Epinephrine	Adrenal medulla	1) Enhances release of glucose from glycogen; 2) Enhances release of fatty acids from adipose tissue.	Raises
Cortisol	Adrenal cortex	1) Enhances gluconeogenesis; 2) Antagonizes Insulin.	Raises
ACTH	Anterior pituitary	1) Enhances release of cortisol; 2) Enhances release of fatty acids from adipose tissue.	Raises
Growth Hormone	Anterior pituitary	Antagonizes Insulin	Raises
Thyroxine	Thyroid	1) Enhances release of glucose from glycogen; 2) Enhances absorption of sugars from intestine	Raises

Table 2: *Hormones that influence blood glucose level* <sup>[28]</sup>

Glucose is used as an energy source in most organisms, from bacteria to humans and used in glycolysis metabolic pathway. Glucose is also a precursor to other molecules like starch, cellulose and glycogen.





**Figure 6:** *Glycolysis mechanism* <sup>[29]</sup>

Glycolysis is the metabolic pathway that converts glucose,  $C_6H_{12}O_6$ , into pyruvate,  $CH_3COCOO^- + H^+$ . This is a vital chemical cascade such that the free energy released in this process is used to form the high-energy compounds ATP (adenosine triphosphate) and NADH (reduced nicotinamide adenine dinucleotide)

It is also due to this vital importance of blood glucose that it needs to be closely monitored in case of diabetes conditions to prevent danger to the patient's life. Hence the necessity for non-invasive quick working blood glucose monitors.

#### 2.2.2.2 Urea

Urea, also known as carbamide, is an organic waste product of the body produced during protein metabolization. In protein metabolization, proteins and amino acids are broken down by liver into urea, which is then secreted outside of the body either as a component of sweat, or within urine by excretion.

The urea levels in body are closely related to the functioning efficiency of the kidneys, hence used widely by the physicians during check-ups to non-invasively determine how efficiently kidneys are functioning.

The main functioning of urea formation and excretion in the body is to get rid of excess nitrogen within the body.

### 2.2.2.3 Creatinine

Serum creatinine (a blood measurement) is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism. Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle, via a biological system involving creatine, phosphocreatine (creatine phosphate), and adenosine triphosphate (ATP, the body's primary energy supply).

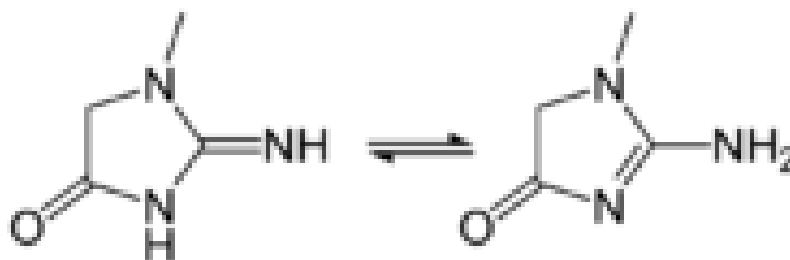


Figure 7: Creatinine<sup>[31]</sup>

### 2.2.2.4 Bilirubin

Bilirubin is the yellow breakdown product of normal heme catabolism. Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism. Bilirubin, when oxidized, reverts to become biliverdin once again. This cycle, in addition to the demonstration of the potent antioxidant activity of bilirubin, has led to the hypothesis that bilirubin's main physiologic role is as a cellular antioxidant.

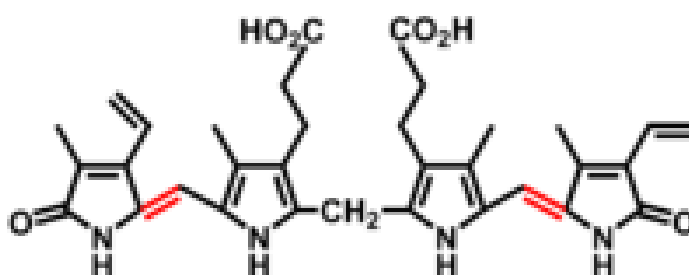


Figure 8: Bilirubin<sup>[32]</sup>

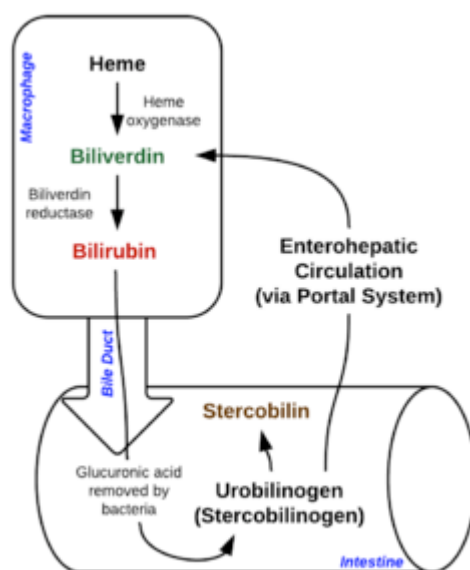


Figure 9 : Bilirubin metabolism <sup>[32]</sup>

#### 2.2.2.5 ALT

Alanine transaminase or ALT is a transaminase enzyme. It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase (ALAT). ALT is found in serum and in various bodily tissues, but is most commonly associated with the liver. It catalyses the two parts of the alanine cycle, the transfer of an amino group from alanine to  $\alpha$ -ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate.

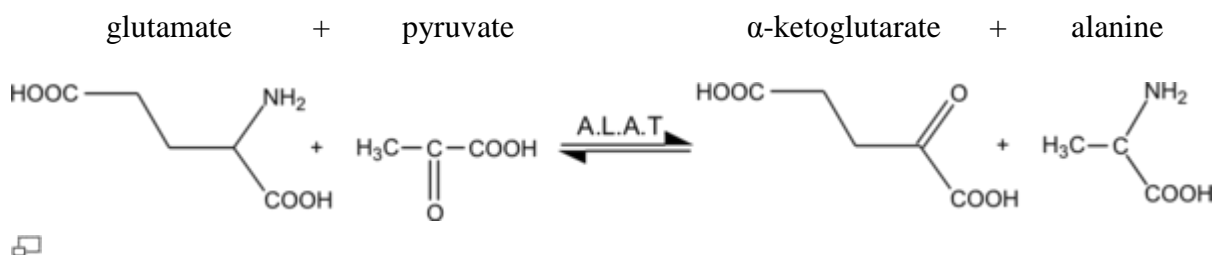


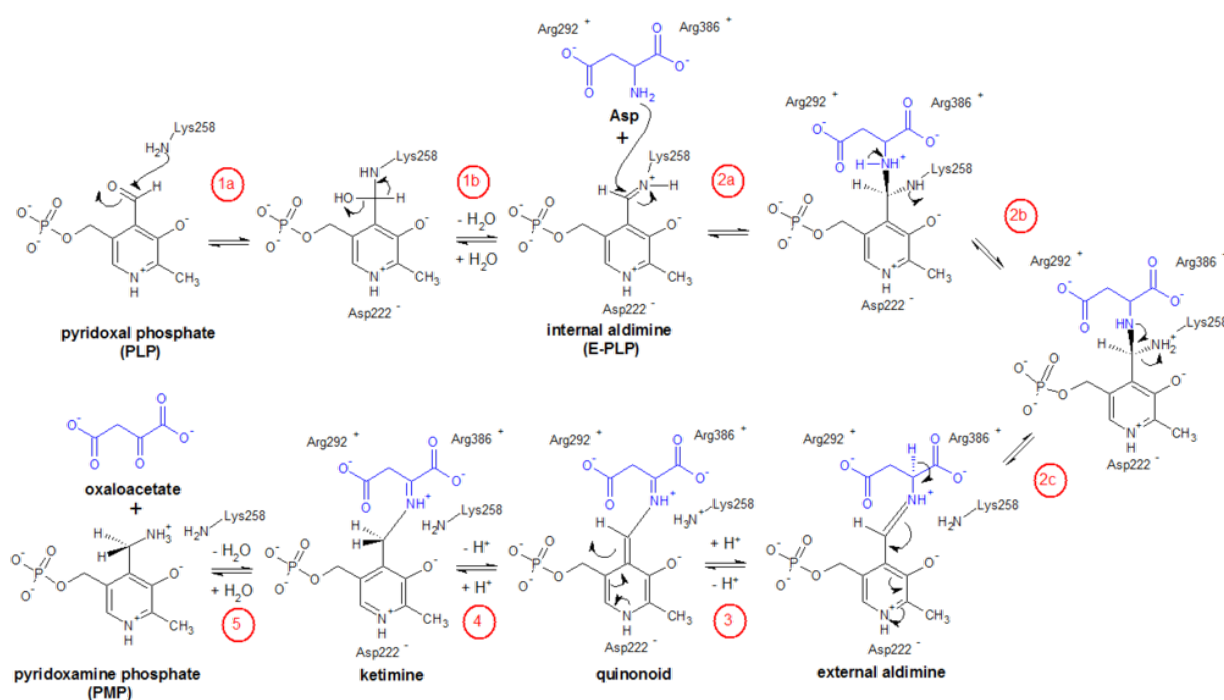
Figure 10: ALT acting as an catalyst in a reaction of glutamate and pyruvate <sup>[33]</sup>

ALT (and all transaminases) requires the coenzyme pyridoxal phosphate, which is converted into pyridoxamine in the first phase of the reaction, when an amino acid is converted into a keto acid.

Significantly elevated levels of ALT(SGPT), 5-38 IU/L for females and 10-50 IU/L for males, often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems. Elevated ALT may also be caused by dietary choline deficiency.

### 2.2.2.6 AST

Aspartate transaminase (AST), also called aspartate aminotransferase (AspAT/ASAT/AAT) or serum glutamic oxaloacetic transaminase (SGOT) is a pyridoxal phosphate (PLP)-dependent transaminase enzyme. AST catalyzes the reversible transfer of an  $\alpha$ -amino group between aspartate and glutamate and, is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health.



**Figure 11:** Reaction mechanism for aspartate aminotransferase <sup>[34]</sup>

### **3. Principles of non-invasive measurements**

The idea of a non-invasive measurement is to obtain information of a certain chemical or mineral in blood without the need to take it outside the body artificially. These kinds of measurements are usually carried out by penetrating the skin by some kind of radiation and measuring the gathering information by taking measurements of the reflected or refracted waves. This type method may also involve making use of naturally excreted by-products of the body, or bodily fluids secreted outside body, like tears or sweat for laboratory analysis.

The need for these kinds of measurements was brought about by the necessity to take continuous measurements of a certain condition of the patient, especially in case of conditions that require continuous monitoring and regulation. There are plenty of advantages in carrying out such a method. Firstly, the most fundamental one is the reduced risk of infection. Avoiding the breach of the skin is one of the most efficient ways of protection against infection. Secondly, one can take measurements of desired biochemical or mineral over and over within short intervals without the risk of endangering the patient in any way. Carrying out a biochemical panel each time from drawn blood would mean that for each measurement a new sample needs to be drawn from the patient. This factor introduces a limit to how many successive times the test can be carried out and introduces a certain interval in-between each test. Moreover, non-invasive methods enable, in most cases, to obtain data in real time with short delays or sometimes almost none at all.

The disadvantage of non-invasive measurements is that they are good approximations of the actual values, however, not as accurate as invasive methods. However, considering cost and benefits factors, non-invasive methods are preferred in most cases with urgency to obtain results.

Out of all the vital minerals and biochemical components of blood, we will be focusing on blood glucose measurement techniques because about 85% of world's biosensor market is accounted by blood glucose measuring devices. This is equivalent to a monetary value of about \$5 billion.<sup>[11]</sup> There being such a gigantic market for blood glucose measuring devices means that there is also an enormous demand for these devices. This is also consistent with the fact that there are over 170 million diabetic patients over the world (according to a research in 2004) and this population is growing fast, increasing the demand for such devices even more.

<i>Statistics</i>	<i>1995</i>	<i>2000 (estimate)</i>	<i>2025 (estimate)</i>
<b>People with Diabetes (million)</b>	<b>135</b>	<b>154</b>	<b>300</b>
<b>% in Developed Countries</b>	<b>25.2</b>	<b>23.8</b>	<b>17.1</b>
<b>Female/Male Ratio</b>	<b>1.18</b>	<b>1.16</b>	<b>1.12</b>

*Source: World Health Organisation*

Table 3: *An estimation of how the population of diabetic patients will increase in the following years.* <sup>[11]</sup>

The blood glucose measurement techniques are divided into three major groups. These are invasive methods, minimally invasive methods and non-invasive methods.

## 4. Types of blood glucose measurements

### 4.1 Invasive Methods

These methods include the first discovered methods to measure blood glucose levels. They involve drawing a blood sample from the individual and testing it with chemical reagents or enzymes, biological reagents in laboratory and glucometers.

#### 4.1.1 Chemical & Biochemical Methods

The blood sample drawn is tested in the laboratory with either chemical reagents or enzymes. From these reactions and end-product concentration analysis, the blood glucose levels can easily be calculated.

I. CHEMICAL METHODS		
A. Oxidation-reduction reaction		
Glucose + Alkaline copper tartarate $\xrightarrow{\text{Reduction}}$ Cuprous oxide		
1. Alkaline copper reduction		
Folin-Wu method	$\text{Cu}^{++} + \text{Phosphomolybdic acid} \xrightarrow{\text{Oxidation}} \text{Phosphomolybd}$	Blue end-product
Benedict's method	<ul style="list-style-type: none"><li>Modification of Folin-Wu method for qualitative urine glucose</li></ul>	
Nelson-Somogyi method	$\text{Cu}^{++} + \text{Arsenomolybdic acid} \xrightarrow{\text{Oxidation}} \text{Arsenomolybdent}$	Blue end-product
Neocuproine method	$\text{Cu}^{++} + \text{Neocuproine} \xrightarrow{\text{Oxidation}} \text{Cu}^{++} \text{neocuproine complex}$ *	Yellow-orange color neocuproine
Shaeffer-Hartmann-Somogyi	<ul style="list-style-type: none"><li>Uses the principle of iodine reaction with cuprous byproduct.</li><li>Excess I<sub>2</sub> is then titrated with thiosulfate.</li></ul>	
2. Alkaline Ferricyanide Reduction		
Hagedorn-Jensen	Glucose + Alkaline ferricyanide $\longrightarrow$ Ferrocyanide	Colorless end product; other reducing substances interfere

		with reaction
B. Condensation		
Ortho-toluidine method	<ul style="list-style-type: none"> <li>• Uses aromatic amines and hot acetic acid</li> <li>• Forms Glycosylamine and Schiff's base which is emerald green in color</li> <li>• This is the most specific method, but the reagent used is toxic</li> </ul>	
Anthrone (phenols) method	<ul style="list-style-type: none"> <li>• Forms hydroxymethyl furfural in hot acetic acid</li> </ul>	
II. ENZYMATIC METHODS (BIOLOGICAL METHODS)		
A. Glucose oxidase		
$\text{Glucose} + \text{O}_2 \xrightarrow[\text{Oxidation}]{\text{glucose oxidase}} \text{Cuprous oxide}$		
Saifer-Gerstenfeld method	$\text{H}_2\text{O}_2 + \text{O-dianisidine} \xrightarrow[\text{Oxidation}]{\text{peroxidase}} \text{H}_2\text{O} + \text{oxidized chromog}$	Inhibited by reducing substances like BUA, bilirubin, glutathione, ascorbic acid
Trinder method	<ul style="list-style-type: none"> <li>• uses 4-aminophenazone oxidatively coupled with phenol</li> <li>• Subject to less interference by increases serum levels of creatinine, uric acid or hemoglobin</li> <li>• Inhibited by catalase</li> </ul>	
Kodak Ektachem	<ul style="list-style-type: none"> <li>• A dry chemistry method</li> <li>• Uses reflectance spectrophotometry to measure the intensity of color through a lower transparent film</li> </ul>	
Glucometer	<ul style="list-style-type: none"> <li>• Home monitoring blood glucose assay method</li> <li>• Uses a strip impregnated with a glucose oxidase reagent</li> </ul>	
B. Hexokinase		
$\text{Glucose} + \text{ATP} \xrightarrow[\text{Phosphorylation}]{\text{Hexokinase} + \text{Mg}^{++}} \text{G-6PO}_4 + \text{ADP}$ $\text{G-6PO}_4 + \text{NADP} \xrightarrow[\text{Oxidation}]{\text{G-6PD}} \text{G-Phosphogluconate} + \text{NADPH} + \text{H}^+$		
<ul style="list-style-type: none"> <li>• NADP as cofactor</li> <li>• NADPH (reduced product) is measured in 340 nm</li> </ul>		



- More specific than glucose oxidase method due to G-6PO<sub>4</sub>, which inhibits interfering substances except when sample is hemolyzed

**Table 4:** *Summary of reactions glucose undergoes in presence of different chemical reagents or enzymes* <sup>[13]</sup>

#### 4.1.2 Glucometer

“A glucose meter (or glucometer) is a medical device for determining the approximate concentration of glucose in the blood.” “A small drop of blood, obtained by pricking the skin with a lancet, is placed on a disposable test strip that the meter reads and uses to calculate the blood glucose level. The meter then displays the level in mg/dl or mmol/l.”<sup>[14]</sup>



**Figure 12:** *Four generations of blood glucose meter, c. 1993-2005. Sample sizes vary from 30 to 0.3  $\mu$ l. Test times vary from 5 seconds to 2 minutes (modern meters typically provide results in 5 seconds).* <sup>[14]</sup>

## 4.2 Minimally Invasive Methods

### 4.2.1 Reverse Iontophoresis (GlucoWatch)

The GlucoWatch Biographer uses reverse iontophoresis to extract glucose across the skin by electro-osmotic flow to monitor glycemia in diabetes. This method uses invasive daily calibration with a conventional “fingerstick”, hence is a commonly used minimally invasive method.

This disadvantage of being invasive has been addressed by using the sodium ion as an internal standard to make this method completely non-invasive. Please refer to the following sections for more information on reverse iontophoresis as non-invasive methods.

### 4.2.2 Microdialysis Probe

Microdialysis is a minimally-invasive sampling technique that is used for continuous measurement of free, unbound analyte concentrations in the extracellular fluid of virtually any tissue. The microdialysis technique requires the insertion of a small microdialysis catheter (microdialysis probe) into the tissue of interest. The microdialysis probe is designed to mimic a blood capillary and consists of a shaft with a semipermeable hollow fiber membrane at its tip, which is connected to inlet and outlet tubing. The probe is continuously perfused with an aqueous solution (perfusate) that closely resembles the (ionic) composition of the surrounding tissue fluid at a low flow rate of approximately 0.1-5 $\mu$ L/min. Once inserted into the tissue or (body) fluid of interest, small solutes can cross the semipermeable membrane by passive diffusion. The direction of the analyte flow is determined by the respective concentration gradient and allows the usage of microdialysis probes as sampling as well as delivery tools. The solution leaving the probe (dialysate) is collected at certain time intervals for analysis.

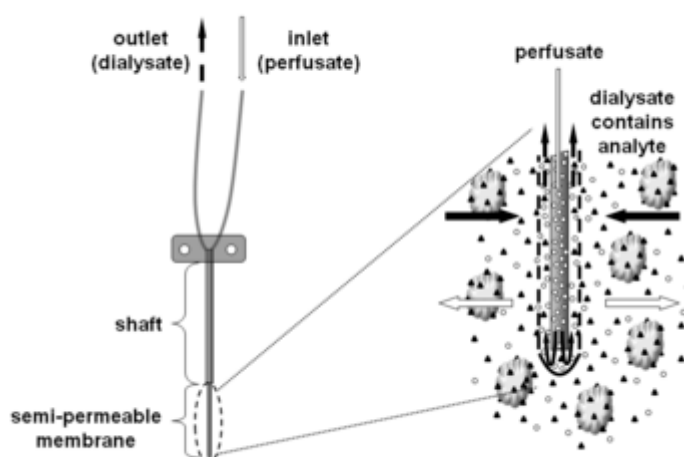


Figure 13: *Microdialysis probe*

### 4.2.3 Fluorescent Sensor

Fluorescent glucose biosensors are devices that measure the concentration of glucose in diabetic patients by means of sensitive protein that relays the concentration by means of fluorescence, an alternative to amperometric sensing of glucose. No device has yet entered the medical market; however, some glucose sensitive fluorescent agents have been successfully developed.

### **4.3 Non-invasive methods**

Non-invasive blood glucose monitoring involves either radiation or fluid extraction<sup>1</sup>. The most promising radiation technologies are: 1) mid-infrared radiation (Mid-IR) spectroscopy; 2) near-infrared radiation (Near-IR) spectroscopy; 3) radio wave impedance; and 4) optical rotation of polarized light.

#### **4.3.1 Optical Techniques**

##### **4.3.1.1 Mid-infrared Spectroscopy**

Mid-infrared (Mid-IR) spectroscopy is based on light in the 2500–10,000 nm spectrum. The physical principle is similar to that of NIR. When compared to NIR, however, due to the higher wavelengths, Mid-IR exhibits decreased scattering phenomena, and increased absorption. For this reason, the tissue penetration of light can reach a few micrometers: in the case of human skin, that corresponds to the stratum corneum. As a consequence, only reflected, scattered light can be considered: there is no light transmitted through a body segment. On the other hand, a possible advantage of Mid-IR compared to NIR is that the Mid-IR bands produced by glucose, as well as other compounds, are sharper than those of NIR, which are often broad and weak.

One strong limitation of this method is the poor penetration. Furthermore, Mid-IR is affected by similar problems and confounding factors than NIR, despite glucose bands potentially improved. For instance, some studies have shown significant dependence of skin Mid-IR spectrum on its water content.

Mid-IR is less studied technique compared to NIR for glucose measurement, probably due to the strong limitation in penetration. Studies are reported related to finger skin and oral mucosa.

##### **4.3.1.2 Fluorescence**

This technique is based on the generation of fluorescence by human tissues when excited by lights at specific frequencies. In the case of glucose, one study demonstrated that when a glucose solution is excited by an ultraviolet laser light at 308 nm, fluorescence can be detected at 340, 380, 400 nm, with maximum at 380 nm. It was also proved that fluorescence intensity was dependent upon glucose concentration in the solution. Also light in the visible spectrum can be used, but this is more adequate for studying fluorescence of tissues rather than that of solutions.

In tissues, the use of ultraviolet light could lead to strong scattering phenomena, in addition to fluorescence. Moreover, even when using different wavelengths, the fluorescence phenomenon can depend not only on glucose, but on several parameters, such as skin pigmentation, redness, and epidermal thickness.

In humans, estimations of glucose concentration have been attempted on skin with patented blue light fluorescence technology.

#### **4.3.1.3 Time of Flight**

Time of Flight (TOF) measurement method has been adopted to measure the effect of glucose on blood in vitro at a wavelength of 906 nm. In photon migration measurements with the TOF technique, short laser pulses are injected into the sample. The photons of the pulse undergo many absorption and scattering events when traveling in the sample. The scattering processes make the photon path lengths longer. Useful data can be obtained about the optical properties of the sample ( $\mu_s$  and  $\mu_a$ ) by observing the TOF distributions of the photons and by analysing their shapes. The calculation of different pulse parameters, such as mean time-of-flight, full width at half maximum (FWHM), integral of the pulse, center-of-gravity, and moments may help in this analysis. A picosecond (ps) laser module with an approximately 30 ps pulse length at a wavelength of 906 nm was used as a light source and a streak camera was used as a detector in the laser pulse measurements. The energy of a pulse is 1 nJ. The detection of photons in the streak camera is done with a photocathode of the streak tube. The light of the photocathode is converted into electrons. The electrons travel through sweep electrodes, which direct them to the microchannel plate (MCP). The electrons are multiplied by the MCP and are eventually converted into light on a phosphor screen. The image on the phosphor screen, containing intensity information as a function of time, is captured with a CCD camera and shown on a computer screen. The TOF technique with a streak camera takes a long measurement time.

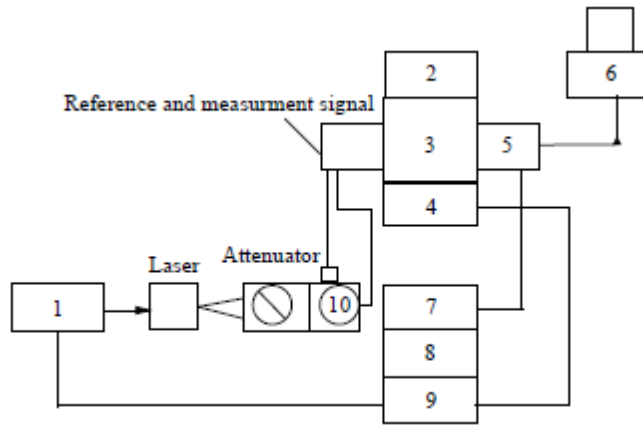


Figure 14: Time-of-flight measurement system. <sup>[51]</sup>

1: Picosecond laser module, 2: Blanking unit, 3: Fastspeed sweep unit, 4: Streak camera, 5: Digital camera, 6: PC, 7: Camera controller, 8: Power supply unit, and 9: Delay unit

#### 4.3.1.4 Near infrared Spectroscopy

Near infrared (NIR) spectroscopy technique is based on the body being focused onto by a beam of light in the 750–2500 nm spectrum. NIR spectroscopy allows glucose measurement in tissues in the range of 1–100 mm of depths, with a decrease in penetration depth for increasing wavelength values. The light focused on the body is partially absorbed and scattered, due to its interaction with the chemical components within the tissue. Attenuation of light in tissue is described, according to light transport theory, by the equation  $I = I_0 e^{-\text{meff}d}$ , where  $I$  is the reflected light intensity,  $I_0$  is the incident light intensity,  $\text{meff}$  is the effective attenuation coefficient and  $d$  is the optical path length in tissue. On the other hand,  $\text{meff}$  can be expressed as a function  $\text{meff} = f(\text{ma}, \text{ms})$ , where  $\text{ma}$  is the absorption coefficient and  $\text{ms}$  is the scattering coefficient. Changes in glucose concentration can influence  $\text{ma}$  of a tissue through changes of absorption corresponding to water displacement or changes in its intrinsic absorption. Changes in glucose concentration also affect the intensity of light scattered by the tissue, i.e.  $\text{ms}$ . This coefficient is a function of the density of scattering centers in the tissue observation volume, the mean diameter of scattering centers, their refractive index and the refractive index of the surrounding fluid. For the case of cutaneous tissue, connective tissue fibers are the scattering centers. Erythrocytes are the scattering centers for blood. In short, glucose concentration could be estimated by variations of light intensity both transmitted through a glucose containing tissue and reflected by the tissue itself. Transmission or reflectance (localized or diffuse) of the light can be measured by proper detectors.

There are limitations associated with this method. The absorption coefficient of glucose in the NIR band is low and is much smaller than that of water by virtue of the large disparity in their respective concentrations. Thus, in the NIR the weak glucose spectral bands only overlap with the stronger bands of water, but also of haemoglobin, proteins and fats. As regards the scattering coefficient, the effect of a solute (like glucose) on the refractive index of a medium is non-specific, and hence it is common to other soluble analytes. Furthermore, physical and chemical parameters, such as variation in blood pressure, body temperature, skin hydration, triglyceride and albumin concentrations may interfere with glucose measurement. Errors can also occur due to environmental variations, such as changes in temperature, humidity, carbon dioxide and atmospheric pressure. Changes in glucose by themselves can introduce other confounding factors, such as hyperglycemia, as well as hyperinsulinemia (often connected to the former in obese patients) can induce vasodilatation, which results in increased perfusion. This phenomenon increases light absorption, and hence it can lead to errors in the estimation of the blood glucose concentration if not taken into account. Hyperglycemia can also have effects on skin structural properties. Diabetic subjects in fact can exhibit “thick skin” and “yellow skin”, probably due to accelerated collagen aging and elastic fiber fraying. Thus, light reflected from the skin of diabetic subjects may have different intensity than in healthy subjects at equal level of glycemia. Also thermal properties of the skin were found to be different in subjects with hyperglycemia, thus affecting the localized reflectance of light. Hyperglycemia can also cause differences in the refractive index of red blood cells, thus leading to different light scattering. Another confounding factor is due to the fact that NIR measurements often reflect glucose concentration in different body compartments, that is, not only blood but also the interstitial fluid in different body tissues can contribute to the measured signal leading to further errors while using this method.

As for measurement sites, NIR light transmission or reflectance has been studied through an ear lobe, finger web and finger cuticle, skin of the forearm, lip mucosa, oral mucosa, tongue, nasal septum, cheek, arm. NIR diffuse reflectance measurements performed on the finger showed a correlation with blood glucose but predictions were often not sufficiently accurate to be clinically acceptable. Diffuse reflectance studies of the inner lip also showed good correlation with blood glucose and indicated a time lag of some minutes between blood glucose and the measurement signal. Salivary glucose levels did not reflect blood glucose levels. These locations were selected for some advantages, such as high vascularization, little fatty tissue, homogeneous composition, limited temperature variations. It must be noted that, depending on the considered site, some specific intervals in the NIR band have been considered and the choice of one specific site also influenced the type of studied light, i.e. transmitted, or reflected (localized or diffuse), or both.

#### 4.3.1.5 Raman Spectroscopy

The Raman spectroscopy is based on the use of a laser light to induce oscillation and rotation in molecules of one solution. The consequent emission of scattered light is influenced by this molecules vibration, which depends on the concentration of the solutes in the solution. Therefore, it is possible to derive an estimation of glucose concentration in human fluids where glucose is present. The Raman spectrum usually considered is in the interval  $200\text{--}1800\text{ cm}^{-1}$ . In this band, Raman spectrum of glucose is quite clearly differentiable from that of other compounds. In fact, Raman spectroscopy usually provides sharper and less overlapped spectra compared, for instance, to NIR. Other advantages are the modest interference from luminescence and fluorescence phenomena. Fixed wavelength lasers at relatively low cost can be used. Recently, an improvement in traditional Raman spectroscopy has been proposed (surface-enhanced Raman spectroscopy), which may increase the sensitivity of the acquisition and/or decreasing the acquisition time.

Main limitations are related to instability of the laser wavelength and intensity, and long spectral acquisition times. Moreover, similarly to other techniques described before, the problem of the interference related to other compounds remains.

The eye is the most common measurement site for this technique. The laser light is passed tangentially through the front of the eye. Another possible site is human skin, although more confounding compounds, such as lipids, are present.

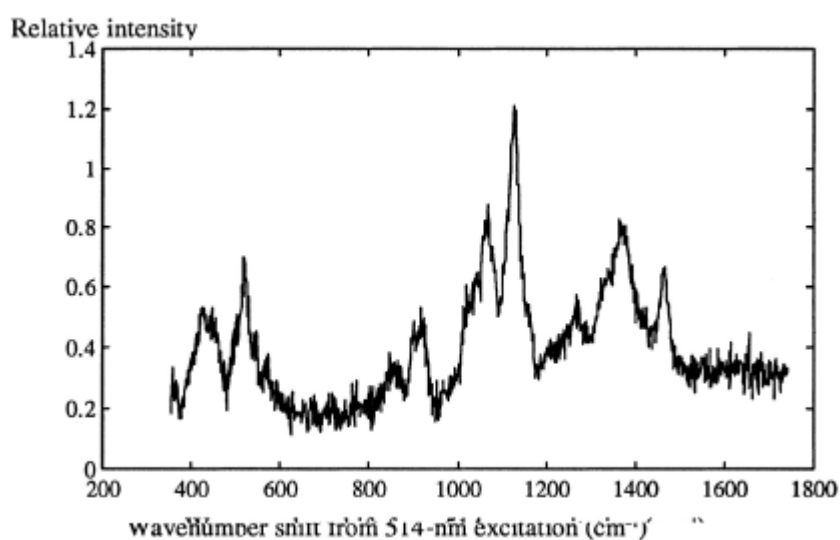


Figure 15: *Raman Spectroscopic Image.* <sup>[51]</sup>

Typical Stokes Raman spectrum is shown for glucose as a vibrational intensity versus shift in wave numbers from the 514-nm excitation wavelength. The water background has been subtracted and the background sample auto fluorescence has also been removed for this nearly saturated glucose concentration.

#### **4.3.1.6 Photoacoustic Techniques**

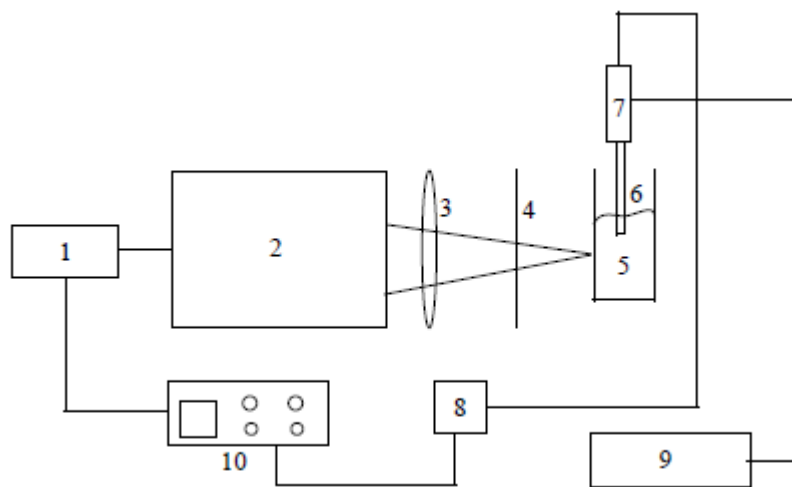
The most used technology based on ultrasound is the photoacoustic spectroscopy, which is based on the use of a laser light for the excitation of a fluid and consequent acoustic response. The fluid is excited by a short laser pulse (from pico to nanoseconds), with a wavelength that is absorbed by a particular molecular species in the fluid. Light absorption causes microscopic localized heating in the medium, which generates an ultrasound pressure wave that is detectable by a microphone. In clear media (that is, optically thin), the photoacoustic signal is a function of the laser light energy, the volume thermal expansion coefficient, the speed of sound in the fluid, the specific heat and the light absorption coefficient. In these media, the signal is relatively unaffected by scattering. The photoacoustic spectrum as a function of the laser light wavelength mimics the absorption spectrum in clear media. However, this technique can provide higher sensitivity than traditional spectroscopy in the determination of glucose. This is also due to the relatively poor photoacoustic response of water, which makes easier the determination of compounds, such as hydrocarbons and glucose. The laser light wavelengths that can be used vary in a wide interval (from ultraviolet to NIR). One variation in photoacoustic spectroscopy is based on combining it with ultrasounds emission and detection. A possible approach is the use of an ultrasound transducer to locate a bolus of blood in a vessel and then illuminate it with the laser pulse at a glucose absorption wavelength. The ultrasound transducer also detects the generated photoacoustic signal. Another approach is detecting ultrasound signal reflected from a blood vessel before and after photoacoustic excitation. Glucose is then determined from the difference in reflected ultrasound intensity. A third approach is exciting a blood bolus via photoacoustic effect: the change in the dimensions and speed of the excited bolus causes a Doppler shift in an ultrasound directed towards the blood vessel; glucose is determined from the magnitude and the delay of the Doppler-shifted ultrasound peak. Finally, a different approach is using ultrasound, possibly coupled with other techniques, but without any photoacoustic effect.

This technique is sensitive to chemical interferences from some biological compounds and to physical interferences from temperature and pressure changes. Moreover, when the laser light transverses a dense media, its contribution to the photoacoustic signal is due not only to the absorption coefficient but also to the scattering coefficient, thus possibly resulting in a



confounding factor if not taken into account. The photoacoustic spectrum of a dense media is similar to the diffuse reflectance spectrum rather than the absorption spectrum. On the other hand, the scattering can enhance the signal, and hence it can provide an advantage if properly considered. A technological disadvantage is that the instrumentation is still custom made, expensive and sensitive to environmental parameters.

A possible body site for measurement is the eye, and, especially, the eye sclera. Other sites are fingers, and forearm, with contribution in glucose determination by blood vessels, by skin and tissues or both.



**Figure 16:** *Photoacoustic measurement system.* <sup>[51]</sup>

1: Laser unit, 2: Laser resonator, 3: Collimating lens, 4: Filter, 5: Cuvette, 6: Acoustic transducer, 7: Photoacoustic preamplifier, 8: Main amplifier, 9: Power unit, and 10: Oscilloscope.

#### **4.3.1.7 Scattering Changes**

##### **4.3.1.7.1 Spatially Resolved Diffuse Reflectance**

The spatially resolved diffuse reflectance technique uses a narrow beam of light to illuminate a restricted area on the surface of section under study. Diffuse reflectance is measured at several distances from the illuminated area. The intensity of this reflectance depends on both the scattering and absorption coefficients of the tissue. Reflectance measured in the immediate vicinity of the illuminated point is mainly influenced by scattering of the skin, while reflectance farther away from the light source is affected by both scattering and the absorption properties of skin. The recorded light intensity profiles are used to calculate the absorption coefficient ( $\mu_a$ ) and the reduced scattering coefficient ( $\mu_s$ ) of the tissue based on the diffusion theory of light

propagation in tissue. Because  $\mu_s$  and glucose concentration are correlated, the latter can be extracted by observing changes in the former. Brulsema et al [35] carried out a glucose clamp experiment to measure the diffuse reflectance. An optical probe was fixed on the patient's abdomen. The clamping protocol consisted of a series of step changes in blood glucose concentration from the normal level of 5 mM to 15 mM and back to 5 mM. Three different clamping experiments were done on the body of diabetic volunteers at wavelength of 650 nm. The corresponding changes in  $\mu_s$  were estimated to be about -0.20 percent/mM, -0.34 percent/mM and -0.11 % /mM, respectively. A qualitative correlation between the estimated change in  $\mu_s$  and the change in blood glucose concentration was observed in 30 out of 41 diabetic volunteers. Heinemann [35] obtained a similar result (-1.0 % / 5.5 mM) in their glucose clamp experiments. However, under normal conditions, blood glucose concentration does not change as rapidly as the clamping experiments suggest. Hence, OGTT was applied in the measurement of diffuse reflectance in reference [35] at the wavelength of 800 nm. The results indicated a mean relative change in  $\mu_s$  of about -0.5 %/mM and 0.3%/mM for healthy persons and type II diabetic patients, respectively. The acceptable correlation between blood glucose concentration and  $\mu_s$  was 75% (27 out of 36 measurements). The recent results by Khalil et al. [36] measured with the spatially resolved diffuse reflectance technique show that temperature affects the cutaneous scattering coefficient  $\mu_s$  and absorption coefficient ( $\mu_a$ ) values. Cutaneous  $\mu_s$  shows linear changes as a function of temperature whereas the changes in ( $\mu_a$ ) showed complex and irreversible behaviour. The thermal response of skin has been used as a basis for non-invasive differentiation of normal and diabetic skin [37]. Recently Poddar et al [38] shown a logarithmic correlation between BGC and  $\mu$ 's. They interpret data in terms of Monte-Carlo simulation to find values of  $\mu$ 's,  $\mu_a$  and  $g$ .

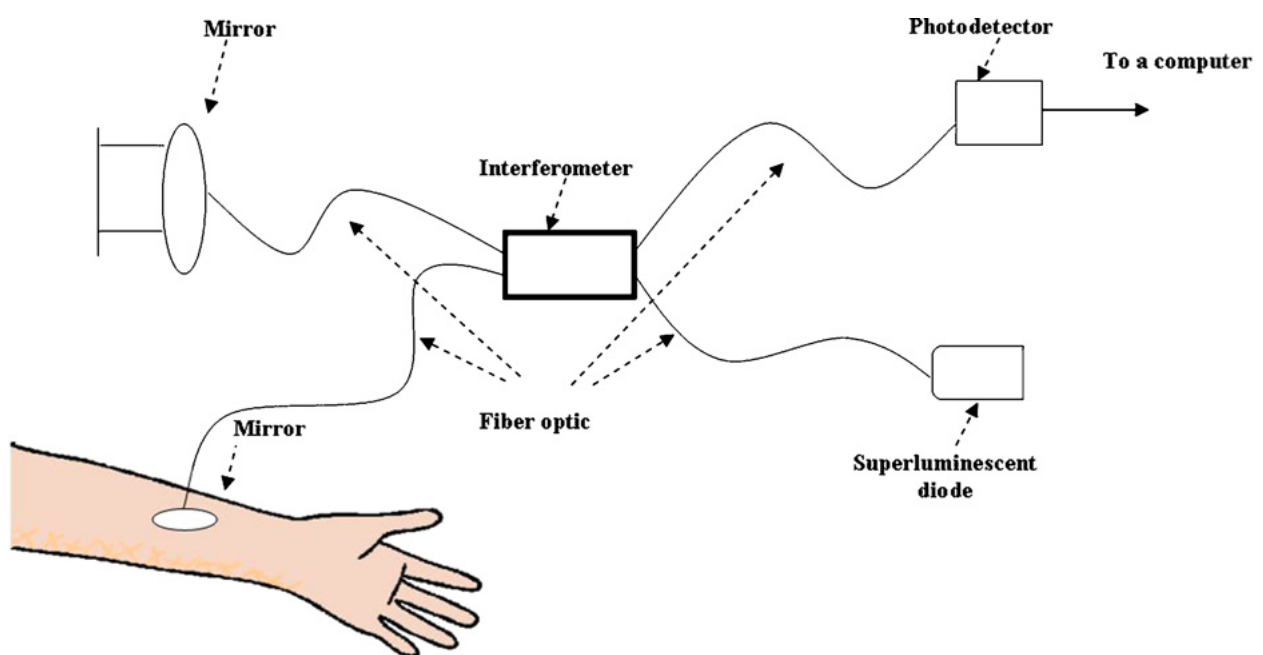
#### **4.3.1.7.2 Optical Coherence Tomography**

The optical coherence tomography (OCT) is based on the use of a low coherence light, such as a super-luminescent light, an interferometer with a reference arm and a sample arm, a moving mirror in the reference arm and a photo-detector to measure the interferometric signal. Light backscattered from tissues is combined with light returned from the reference arm of the interferometer, and the resulting interferometric signal is detected by the photo-detector. What is measured is the delay correlation between the backscattered light in the sample arm and the reflected light in the reference arm. A block diagram of the experimental system is reported in Figure 17. Moving the mirror in the reference arm of the interferometer allows scanning the tissues up to a depth of about 1 mm. By moving the mirror into the sample arm scanning of the

tissue surface is obtained. Therefore, this technique has unique capability of in-depth and lateral scanning to obtain two-dimensional images with high resolution. Tissue scattering properties are highly dependent on the ratio of the refractive index of scattering centers (cellular components, proteins, etc.) to the refractive index of the interstitial fluid. An increase of glucose concentration in the interstitial fluid causes an increase in its refractive index, thus determining a decrease in refractive index mismatch, and hence of the scattering coefficient. Therefore, from the OCT data, generated by the backscattered light, it is possible to get an estimation of glucose concentration in the interstitial fluid. It must be noted that OCT technique is similar to the light scatter technique described before, because both techniques exploit the scattering of light. However, in the light scatter technique (localized reflectance) the intensity of collected light is studied, while in OCT the delay of backscattered light compared to the light reflected by the reference arm mirror is considered. However, some studies demonstrated that the effect of glucose on the scattering coefficient values measured by OCTs similar to that measured by light scatter technique.

OCT technique can be sensitive to motion artifacts. Moreover, although little changes in skin temperature have negligible effects, changes of several degrees have a significant influence on the signal. Finally, as already mentioned there is currently no clear indication that this technique has advantages compared to other scattering based techniques.

The body site for measurement is the skin, typically in the forearm. More specifically, glucose concentration in the interstitial fluid of the upper dermis of the skin is investigated.



**Figure 17:** *Optical Coherence Tomography system*<sup>[50]</sup>

#### **4.3.1.8 Polarization Changes**

It is based on the phenomenon that occurs when polarized light transverses a solution containing optically active solutes (such as chiral molecules): the light, in fact, rotates its polarization plane by a certain angle, which is related to the concentration of the optically active solutes. Glucose is a chiral molecule, and its light rotation properties have been known for a long time. Indeed, investigation of the polarization changes induced by glucose is reported to be the first proposed non-invasive technique for glucose measurement in humans. One advantage of this technique is that it can make use of visible light, easily available. Moreover, the optical components can be easily miniaturized.

This technique is sensitive to the scattering properties of the investigated tissue, since scattering depolarizes the light. As a consequence, skin cannot be investigated by polarimetry, since it shows high scattering mainly due to the stratum corneum. Moreover, the specificity of this technique is poor, since several optically active compounds are present in human fluids containing glucose, such as ascorbate and albumin. However, specificity can be partially improved by using multiple light wavelengths. Other general sources of errors are variations in temperature and pH of the solution.

The preferential site for this technique is the eye, and, more specifically, the aqueous humor beneath the cornea. Cornea has in fact low scattering properties, since it does not have the stratum corneum. However, some sources of errors are due to eye movements and corneal rotations. The corneal birefringence, due to its collagen structure, is another error source. Moreover, a time delay between glucose concentration in aqueous humor and blood has been observed, and hence it has to be taken into account.

#### **4.3.1.9 Temperature Modulated |Localized Reflectance**

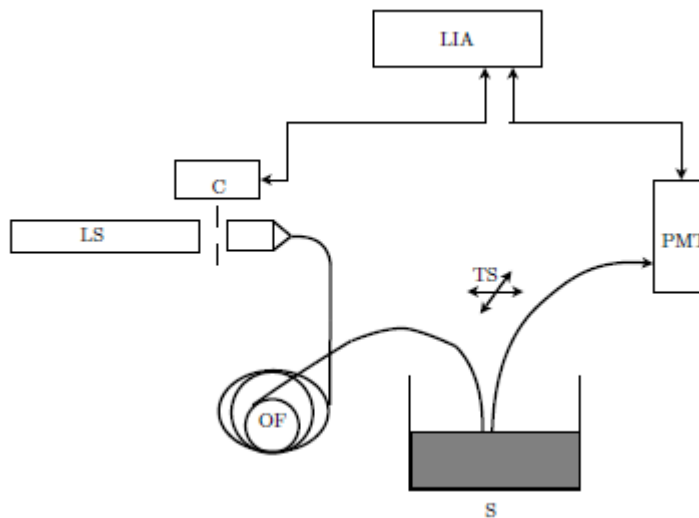
This technique is based on the observation that temperature changes causes variations in the tissue's refractive index (which influences the light scattering), but on the other hand the entity of these changes depend upon glucose concentration. More specifically, the temperature modulation of the localized reflected light due to scattering is analysed. Glucose concentration is estimated with localized reflectance signals at 590 and 935 nm. In some studies, a probe was placed in contact with the skin and the probe temperature was varied between 22 and 38°C. After each variation, skin was equilibrated for some minutes. During each of those intervals some light packets were collected, related to glucose concentration.

There are several parameters can affect this kind of measurements, both physiological and technical, such as the placement position of the probe. Also a peculiar health status, such as

an inflammatory state with possible fever condition, can in fact affect the measurement. Measurements are usually performed on the skin of the forearm. In particular, glucose in the dermis layer is estimated.

#### 4.3.1.10 Frequency Domain Reflectance Technique

The optical system used in frequency-domain reflectance measurements is similar to that used in diffuse reflectance measurements, except for the light source and the detector, which are modulated at a high frequency. Then, the phase and intensity of the photon-density wave generated by the source are measured. Combining these measurements with linear transport theory enables the deduction of  $\mu_a/n$  and  $n\mu_s$ , where  $n$  is the mean refractive index of the tissue. Maier et al [41] applied the frequency-domain tissue spectrometer to do an OGTT for a non-diabetic male. The optical source had a wavelength of 850 nm and the measurement location was muscle tissue in the subject's thigh. The experimental results showed that the relative change of  $\mu_s$  with blood glucose concentration was - 2.5 % / 3.6mM, which is identical with the results obtained by Heinemann [40].



**Figure 18:** Schematic of the experimental setup used (Poddar et al) for collection of backscattered signal. LS - light source, C - Chopper, OF - Optical fiber, TS - stepper motor controlled translation stage, LIA- Lock-in amplifier, S - sample under study and PMT - Photomultiplier tube.<sup>[51]</sup>

#### **4.3.1.11 Fluid Harvesting**

This technique is based on the use of a laser light or ultrasounds to create an array of microscopicholes (micropores) on human skin. The interstitial fluid, containing glucose, tends to migrate through the micropores to a glucose sensor (of traditional type) placed in contact with the skin where the micropores have been created, and hence direct glucose measurement becomes possible. The technique based on laser light is called “biophotonic technique”, whereas that based on ultrasound is also called “sonophoresis”.

No limitations or confounding factors have been explicitly described, except than the possible mismatch between glucose in the interstitial fluid and glucose in blood.

The site for measurement is human skin in general. The arm is probably the most common application site. Due to micropores generation, the technique can also be considered as minimally invasive method.

#### **4.3.2 Other Techniques**

##### **4.3.2.1 Photonic Crystal Glucose Sensing (Ocular Spectroscopy)**

This technique is based on the use of a special contact lens where a hydrogel has been added. In *Domschke et al.* [42], a hydrogel wafer with 7 mm of thickness based on boronic acid derivatives was bonded to the lens. The boronic acid derivative is able to create reversible covalent bonds with glucose, and the phenomenon is influenced by the glucose concentration in tears. When the lens is illuminated by a light source, such as a laser light, the reflected light changes its wavelength, hence its observed colour, depending on the entity of the binding phenomenon, which is related to tear glucose concentration. The light colour changes can be detected by a spectrometer.

There exists a delay between glucose in blood and in tears. Moreover, the use of contact lens, though it can be considered a non-invasive approach, may be uncomfortable to some subjects. Besides, the only measurement site for this method is the eye.

A recent development in this method is the development of a new sensor material could be used for non-invasive glucose sensing in tear fluid. The sensor would be contained in contact lenses or in ocular inserts designed for use under the lower eyelid (See Fig.19). Patients would be able to determine their glucose concentration by viewing the color of the sensing material by use of a compact device that contains a white light source, a mirror, and a color chart. The observed color would indicate the glucose concentration in the patient’s tear fluid. Alternatively, a simple spectral measuring instrument could be used to measure the diffracted wavelength; this instrument would calculate and display the blood glucose concentration. Because tear-fluid

glucose concentrations parallel blood glucose concentrations, a tear-fluid glucose measurement could be used to determine the blood glucose concentration. The recently developed glucose-sensing hydrogel senses glucose through the formation of a supramolecular complex, between glucose, two boronates, two  $\text{Na}^+$  ions, and PEG. This glucose cross-link acts as an additional hydrogel cross-link. The PEG localizes  $\text{Na}^+$  close to the two boronates to form ion pairs to electrostatically stabilize the dianion complex by minimizing the electrostatic repulsion between the two boronates. These glucose-induced hydrogel cross-links increase the elastic-restoring force and shrink the hydrogel volume, which blue shifts the diffraction.



Figure 19: *Photonic Crystal Glucose Sensing from eye tear*

#### **4.3.2.2 Metabolic Heat Confirmation Method (Thermal Spectroscopy)**

This technology does in fact include different approaches. In thermal gradient spectroscopy, it is considered the absorptive effects of glucose on the body naturally emitted infrared (IR) radiation. Such IR absorption effect of glucose is obviously related to its concentration. In some studies, the skin was cooled to approximately  $10^{\circ}\text{C}$  to suppress its absorptive effects.

Every variation of body or tissue temperature is a strong confounding effect. It must be noticed that several factors can induce temperature variations, both physiological, such as the circadian periodicity, and pathological, such as a fever status.

A traditional site for this technique is the skin of the forearm or the finger. However, a different possible site is the ear: a sensor can in fact be inserted in the ear canal to measure the IR radiation emitted by the tympanic membrane.

#### **4.3.2.3 Impedance Spectroscopy**

The impedance of one tissue can be measured by a current flow of known intensity through it. If the experiment is repeated with alternating currents at different wavelengths, the impedance (dielectric) spectrum is determined. The dielectric spectrum is measured in the frequency range of 100 Hz to 100 MHz. Variations in plasma glucose concentration induce in red blood cells a decrease in sodium ion concentration, and an increase in potassium ion concentration. These variations cause changes in the red blood cells membrane potential, which can be estimated by determining the permittivity and conductivity of the cell membrane through the dielectric spectrum. Some problems remain to be clarified, such as the effect of body water content and of dehydration. Moreover, some diseases affecting the cell membrane scan also have an influence that needs to be evaluated. The most known study was performed with a watch-like-device, positioned on the wrist.

#### **4.3.2.4 Electromagnetic Sensing**

Similarly to impedance spectroscopy, this technique detects the dielectric parameters of blood. However, in the former an electric current is used, while in the latter the electromagnetic coupling between two inductors is exploited. The inductors are turned around the medium under study. In an in vitro context, the system can be described by Figure 20. The tubes, simulating the human veins, contain blood. To make the experiment more realistic, the tubes can be covered by gelatine, which roughly simulates the tissues surrounding blood vessels in the in vivo context. The electromagnetic coupling of the two inductors is modified by variations in the dielectric parameters of the solution, for example blood in this case. On the other hand, dielectric parameters of the solution are influenced by glucose, and hence an estimate of glucose concentration can be derived. More specifically, the method is based on the application of a voltage signal,  $V_{in}$ , with proper frequency, to the primary inductor. For electromagnetic coupling a signal,  $V_{out}$ , will be produced at the secondary inductor. The ratio  $V_{outRMS}/V_{inRMS}$ , where RMS indicates the effective value, depends in fact by the glucose concentration. The frequencies used in this technique are in the 2.4–2.9 MHz range. However, depending on the temperature of the investigated medium, there exists an optimal frequency, where the sensitivity to glucose changes reaches the maximum. For instance, at 24°C optimal frequency is 2.664 MHz.



The temperature has a strong effect on the measurement. Because it also influences the optimal investigation frequency, temperature measurement has to be performed and the information properly considered. Furthermore, the blood dielectric parameters depend on several components other than glucose.

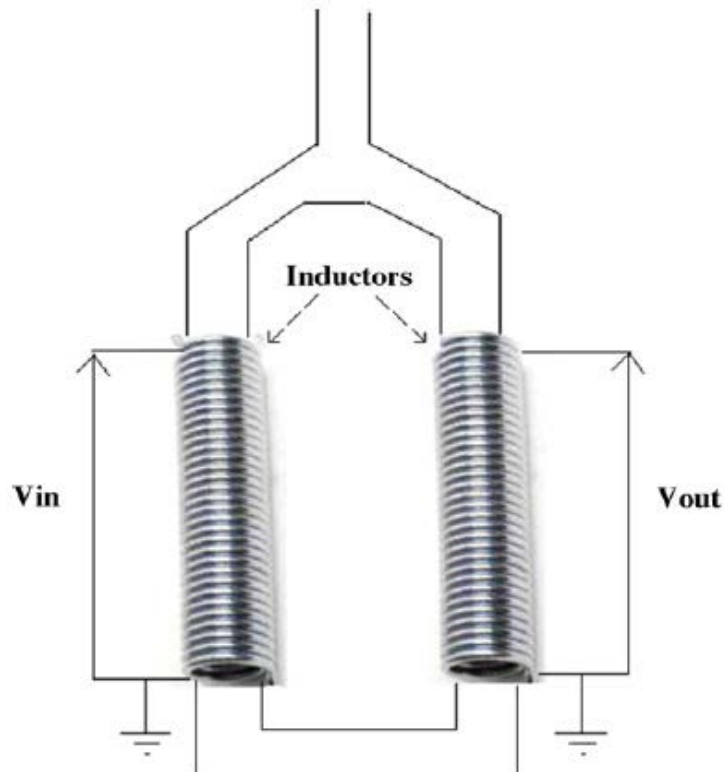


Figure 20: Electromagnetic Sensing System<sup>[50]</sup>

#### 4.3.2.5 Iontophoresis

Iontophoresis (or, more exactly, reverse iontophoresis) is based on the flow of a low electrical current through the skin, between an anode and cathode positioned on the skin surface. An electric potential is applied between the anode and cathode, thus causing the migration of sodium and chloride ions from beneath the skin towards the cathode and anode, respectively. In particular, it is sodium ion migration that mainly generates the current. Uncharged molecules, such as glucose, are carried along with the ions by convective flow (electroosmosis). This flow causes interstitial glucose to be transported across the skin, thus being collected at the cathode where a traditional glucose sensor is placed to get direct glucose concentration measurement. Over the typical range of iontophoretic current densities ( $<0.5 \text{ mA/cm}^2$ ), glucose extraction is approximately in linear relation with the density and duration of iontophoretic current.

The main drawback of this technique is that it tends to cause skin irritation. This problem may be avoided by limiting the time interval of the electrical potential application. On the other hand, a minimum duration is required to get a sufficient amount of glucose for measurement. Furthermore, this approach cannot be used if the subject is sweating significantly. There is also discussion as to whether this technology can be able to detect rapid changes in blood glucose. This is, actually, a general problem of any measurement of glucose not in blood.

The measurement can be obtained with a watch-like-device placed on the wrist. Similarly to the fluid harvesting technique, also iontophoresis is also considered as a minimally invasive. In fact, the glucose is again extracted from the skin and directly measured. The two techniques are sometimes defined as “transdermal” techniques.

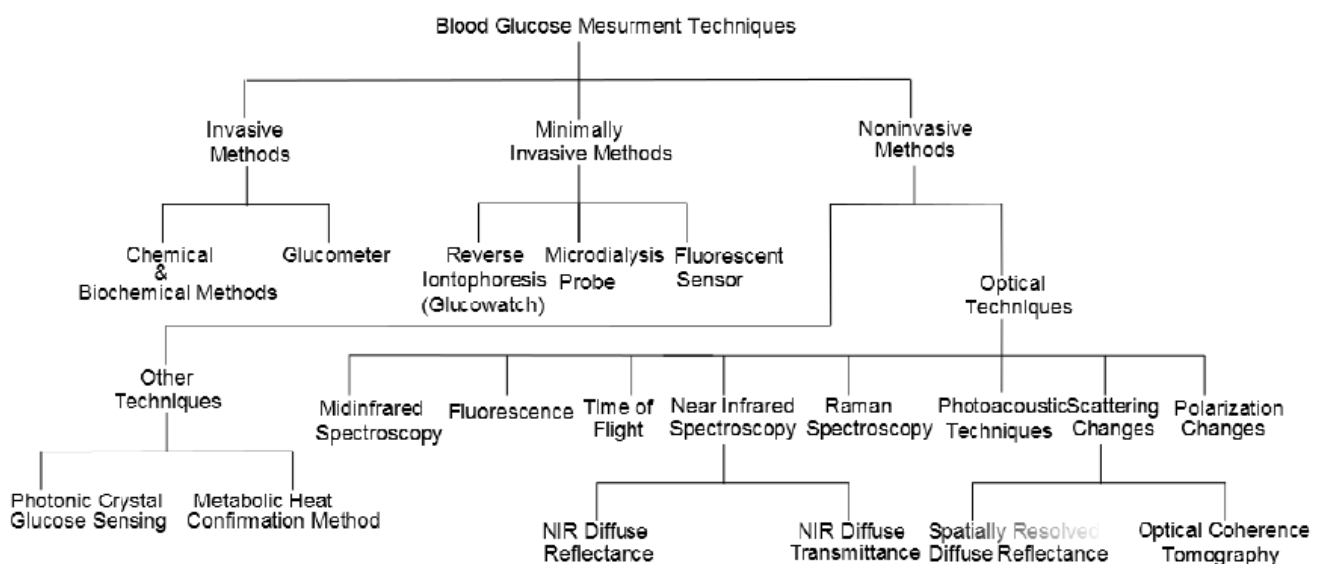


Figure 21: A summary of some of blood glucose measurement techniques. <sup>[12]</sup>

## 5. Types of Devices used for glucose monitoring

### 5.1 Invasive Methods

The devices to be illustrated below are given in an approximately chronological order of being released to the market.

#### 5.1.1 Ames Reflectance Meter



Figure 22: *Ames Reflectance Meter, first glucose meter developed*<sup>[11]</sup>

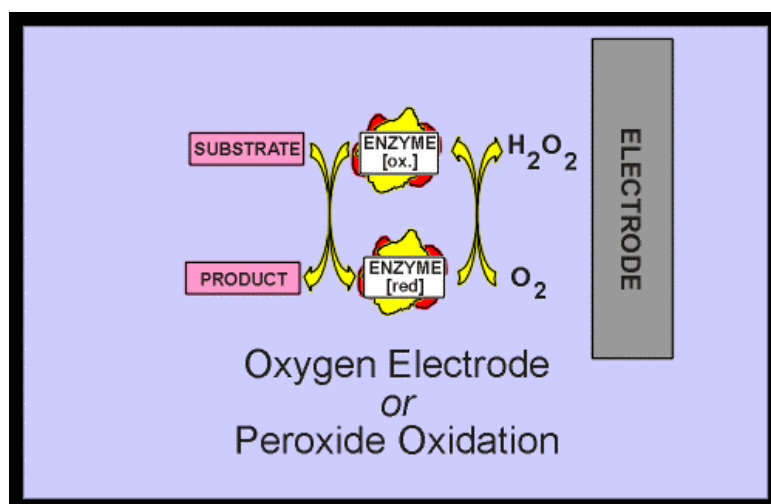


Figure 23: *First Generation Glucose Biosensor Working Principle Schematics* <sup>[11]</sup>

#### 5.1.2 YSI 23A Glucose Biosensor

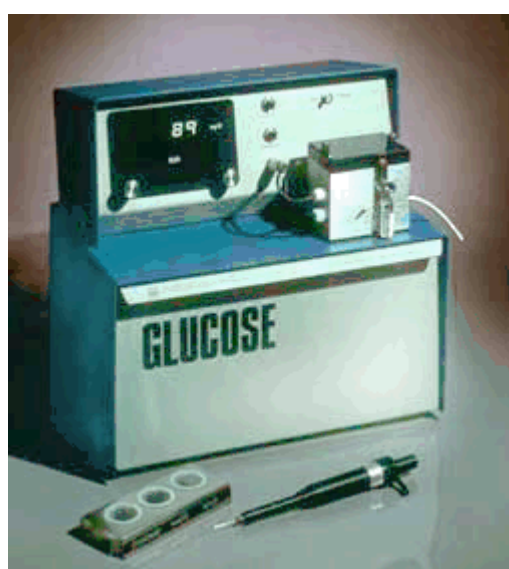


Figure 24: *YSI 23A Glucose Biosensor* <sup>[11]</sup>

### 5.1.3 Laboratory scale screen printer



Figure 25: *Laboratory Scale Screen Printer* <sup>[11]</sup>

Since blood is being handled, a disposable sensor design became preferred. Moreover, minimising the required sample size became a priority. Finger-pricking is uncomfortable, so other sampling sites have been investigated and instrument design has become more automated

### 5.1.4 Commercially available enzyme electrodes



Figure 26: *Commercially available enzyme electrodes* <sup>[11]</sup>

### 5.1.5 Relion Blood Glucose monitor



Figure 27: *Relion Blood Glucose monitor*<sup>[11]</sup>

### 5.1.6 Soft tact monitor



Figure 28: *Soft tact monitor*<sup>[11]</sup>

### 5.1.7 Precision Xtra Glucose/Ketone Monitor



Figure 29: Precision Xtra Glucose/Ketone Monitor <sup>[11]</sup>

### 5.1.8 Ascensia Elite, Elite XL and Breeze Biosensors



Figure 30: Ascensia Elite, Elite XL and Breeze Biosensors <sup>[11]</sup>

### 5.1.9 Ascensia Contour, Microfill Sensors and Dex 2



Figure 31: *Ascensia Contour, Microfill Sensors and Dex 2* <sup>[11]</sup>

### 5.1.10 OneTouch Ultra Blood Glucose Biosensor



Figure 32: *OneTouch Ultra Blood Glucose Biosensor* <sup>[11]</sup>



### 5.1.11 LifeScan UltraSmart System



Figure 33: LifeScan UltraSmart System<sup>[11]</sup>

### 5.1.12 InDuo Combined Meter and Insulin Dosing System



Figure 34: InDuo Combined Meter and Insulin Dosing System<sup>[11]</sup>

### 5.1.13 Accu-Chek Active Meter



Figure 35: Accu-Chek Active Meter<sup>[11]</sup>

#### 5.1.14 Accu-Chek Advantage Glucose Monitor Featuring Comfort Curve Test Strip



Figure 36: *Accu-Chek Advantage Glucose Monitor Featuring Comfort Curve Test Strip*<sup>[11]</sup>

#### 5.1.15 Accu-Chek Compact ☐ Instrument



Figure 37: *Accu-Chek Compact ☐ Instrument*<sup>[11]</sup>

### 5.1.16 MiniMed<sup>®</sup> Implantable Glucose Sensor



Figure 38: *MiniMed<sup>®</sup> Implantable Glucose Sensor* <sup>[11]</sup>

	LifeScan One-Touch Ultra	Roche Diagnostics Accu-Chek Advantage	Bayer Diagnostics Glucometer Elite XL	TheraSense FreeStyle	MediSense Precision Q.I.D
Alternate Site Testing	Yes	No	No	Yes	No
Sample Size (μl)	1	3-4	2	0.3	3.5
Test Time (seconds)	5	40	30	15	20
Capillary Action Strip	Yes	Yes	Yes	Yes	No
Temp. Range (°C)	5 – 44	8 - 39	10 - 39	10 - 35	18 - 30
Test Memory	150	100	120	250	10-125
Data Downloading	Yes	Yes	Yes	Yes	Yes

Table 5: *Gives a comparative analysis of devices illustrated above* <sup>[11]</sup>

## 5.2 Minimally invasive methods

### 5.2.1 Gluowatch

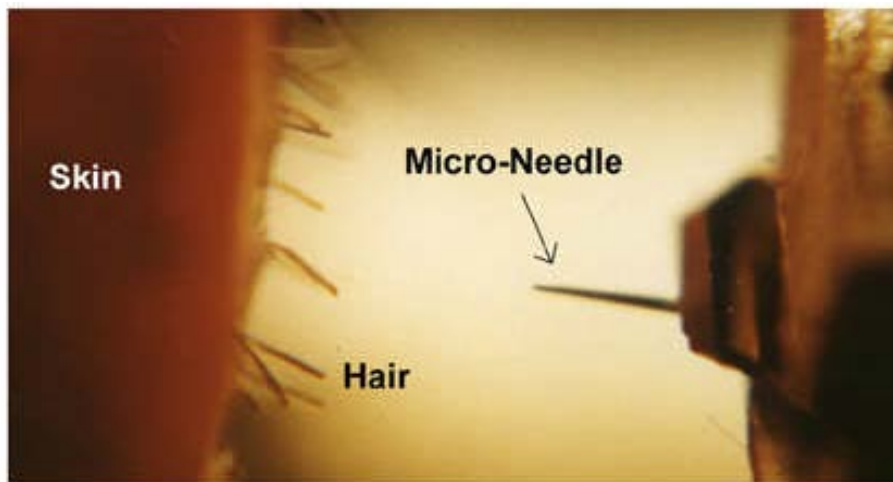


Figure 39: *Gluowatch using micro-needle for minimal invasion* <sup>[11]</sup>

### 5.2.2 Lancet for Obtaining a Sample of Capillary Blood



Figure 40: *Lancet for Obtaining a Sample of Capillary Blood* <sup>[11]</sup>

### 5.2.3 Bayer Microlet® Vaculance



Figure 41: *Bayer Microlet® Vaculance* <sup>[11]</sup>

### 5.2.4 Cell Robotics Lassette® Laser Ablation Blood Sampling Device



Figure 42: *Cell Robotics Lassette® Laser Ablation Blood Sampling Device* <sup>[11]</sup>

Laser ablation uses short laser pulses to open up small pores in the epidermis, allowing a small sample of interstitial fluid to be released (Figure 42). The process is painless and causes minimal damage to the skin.

From the chronological order above, we can observe how the blood glucose measurement devices evolved over the last 50 years.

### 5.3 Non-invasive methods

	Approvals	Technology
GlucoWatch <sup>®</sup> , Cygnus Inc.	CE Mark in 1999. FDA Approval in 2001 for adults only, in 2002 for children aged 7–17 years	Iontophoresis
Diasensor <sup>®</sup> , BICO Inc.	CE Mark in 2000 (for the latest device version)	Near infrared spectroscopy
Pendra <sup>®</sup> , Pendragon Medical Ltd.	CE Mark in 2003	Impedance spectroscopy
Aprise <sup>™</sup> , Glucon Medical Ltd.	The Company is working to achieve FDA Approval. It also plans to achieve CE Mark	Ultrasound technology (photoacoustic spectroscopy)
Glucoband <sup>®</sup> , Calisto Medical Inc.	The device already meets the requirements for FDA Approval: registration is in progress	Impedance spectroscopy
Glucotrack <sup>™</sup> , Integrity Applications Ltd.	CE Mark is expected within 2006, FDA Approval 1 year later	Ultrasound technology
OrSense Ltd.	The Company has the rights over several patents, but approvals are not reached yet	Near infrared spectroscopy
SpectRx Inc.	Not approved yet	Fluid harvesting
SugarTrac <sup>™</sup> , LifeTrac Systems Inc.	A multi-center study is planned to reach FDA approval	Near infrared spectroscopy
Symphony <sup>™</sup> , Sontra Medical Corporation	Sontra claims to start clinical studies for FDA Approval in 2006	Fluid harvesting
Dream Beam, Futrex Medical Instrumentation Inc.	Not approved	Near infrared spectroscopy
GluCall, KMH Co. Ltd.	No information	Iontophoresis
GluControl GC300 <sup>®</sup> , ArithMed GmbH	In 2000, the meter was not approved yet. No recent information found	Near infrared spectroscopy
Hitachi Ltd.	The Company is working to achieve regulatory approval under the Pharmaceutical Affairs Law in Japan and FDA Approval	Thermal spectroscopy
Sysmex Corporation	No information	Unclear (probably iontophoresis)
TouchTrak Pro 200, Samsung Fine Chemicals Co. Ltd.	In 1999 not approved yet	Unclear (probably near infrared spectroscopy)

**Table 6:** *List of devices for measuring blood glucose levels employing non-invasive techniques*<sup>[50]</sup>

### **5.3.1 Meters approved and available**

#### **5.3.1.1 GlucoWatch, Cygnus Inc.**

The meter has a wrist-watch format. It measures glucose through the skin using a disposable pad, which clips into the back of the meter. The pad uses an adhesive to stick to the skin allowing it to come in contact with a small electrical current, which causes the reverse iontophoresis. The electrical charges bring glucose to the skin surface where an enzyme reaction similar to that found in a standard meter occurs. Thus, glucose levels in the interstitial fluid can be estimated. Compared with finger-stick readings, the meter measurements have a 15-min lag time. The meter is intended for use to supplement, but not to replace, information obtained from a standard blood glucose meter. The meter has 2–3 h warm-up period. Afterwards, it measures glucose every 10 min, at least in the latest version, called GlucoWatch G2 Biographer there is a 3 min of electrical stimulation, and then 7 min of glucose measurement. An alarm also occurs when a rapid change of more than a 35% change from any reading in the last hour is seen in the blood sugar, when the subject is sweating, and for any measurements above or below the patient's target levels. A trend indicator appears to show the direction of the blood sugar when the current measurement is more than 18 mg/dl (1 mmol/l) higher or lower than the previous measurements. By the several trials have been carried out on the meter, not only the meter performance were assessed in terms of accuracy, but also different aspects connected to the use of the meter were investigated, such as the long-term effect of using the meter on the glycated hemoglobin compared to traditional finger-stick testing.

There has been various cohort studies and randomized trials carried out with this device. In one of these cohort studies, a clinical trial was performed to assess the effect of acetaminophen (a potential interference for traditional blood glucose meters) on the accuracy of the GlucoWatch in adult subjects with diabetes ( $n = 18$ ). One thousand milligram doses of acetaminophen were administered to the subjects. The GlucoWatch readings were compared to serial finger-stick blood glucose measurements [43]. In another such study, ninety-two subjects with Type 1 or 2 diabetes requiring treatment with insulin were studied. Participants wore up to two GlucoWatch during the 15-h study session and performed two finger sticks per hour for comparative blood glucose measurements [44]. One of the results that came out of the cohort studies was the fact that the mean difference between GlucoWatch and finger stick measurements was -0.01 and 0.26 mmol/l for the clinical and home environments, respectively. The mean absolute value of the relative difference was 1.06 and 1.18 mmol/l for the same studies. Correlation coefficient ( $R$ ) between GlucoWatch and finger-stick measurements was



0.85 and 0.80 for the two studies. In both studies, over 94% of the GlucoWatch readings were in the clinically acceptable A + B region of the Clarke error grid.

The use of the meter is made comfortable by its wrist-watch format. The data download to a PC and subsequent analysis through a proper tool also allows powerful use of the collected data. The meter has a memory that can store up to 8500 records. One limitation is that the meter requires calibration through a standard blood sugar meter. Moreover, the disposable pad must be replaced every 12–13 h of monitoring time to ensure continued accuracy; the meter must then go through the warm-up period and calibration again. In addition, it may take more than one try to calibrate the meter, thus requiring additional finger-stick tests. Occasionally, the meter will not calibrate at all. Some drawbacks have also been documented during the meter use. In fact, the measurements can fail, or be inaccurate, if the patient is sweating, or in the case of rapid temperature changes, excessive movement of the meter, strenuous exercise. Moreover, the user cannot shower, bathe or swim during the warm-up period. Finally, most users report that the electrical discharge is quite noticeable during the first use of the meter, although it becomes less noticeable on subsequent use. Another drawback is that the meter causes skin irritation to some extent. Skin irritation, in fact, limits reuse of the same site to a week or two.

### **5.3.2 Meters approved but withdrawn**

#### **5.3.2.1 Diasensor, BICO Inc.**

Diasensor operates by placing the patient's forearm on the arm tray of the meter. The blood glucose test is obtained in less than 2 min. The dimensions of the meter are relevant compared to other meters, but it is still sufficiently compact to be used in a domiciliary environment. It is claimed that the Diasensor is not for everyone, but it is not clear what patient categories are allowed to use it. In any case, it is not intended as a replacement for the traditional invasive blood glucose meter. Its aim is to promote greater compliance for self-monitoring by allowing patients to perform the majority of their daily blood glucose monitoring with a non-invasive meter. The Diasensor is declared not to be adequate to detect hypoglycaemic events.

A trial was performed where a total of 100 testing sequences were evaluated over a period of more than 1 month. In each sequence, six measurements were performed: the first two with traditional glucose meters, the third with Diasensor and the following again with the 3 meters but in the opposite order. The trial seems to have occurred in late 2000. In another study, the effectiveness of the Diasensor for the management of long-term blood glucose control was evaluated in Type 1 and insulin-requiring Type 2 diabetic patients. Approximately, 200 men and women from nine US medical centers, at least 18 years of age, with Type 1 or insulin-requiring



Type 2 diabetes for at least 1 year, participated in the study. The study lasted about 9 months. It was running in 2002. In the late 2000 trial, the average difference between Diasensor and the traditional meters was around 25%. No results were found on the second trial. Moreover, due to its relatively large size, the device is not portable.

#### **5.3.2.2 Pendra, Pendragon Medical Ltd.**

The meter measures how changes in blood composition affect the impedance pattern of the skin and underlying tissue. In particular, the meter is optimized to detect the effects of glucose on the impedance pattern, so that blood glucose concentration is estimated. The meter has wrist-watch format (53 mm - 46 mm - 15 mm), and it has an open resonant circuit which lies in contact with the skin. This circuit generates small currents in a proper frequency range and performs the impedance measurement. Sensitivity was reported to be in the range of 20–60 mg/dl of glucose per Ohm [45]. The meter can provide up to four measurements per minute, but a new value every minute is displayed (averaged above the measurements in the last 5 min). Alerts are provided in the case of hypoglycemic events, and whether rapid changes of glucose concentration occur. Variations on impedance due to temperature changes are self-corrected. The meter can be used by both Types 1 and 2 diabetic subjects. However, in Type 1 subjects possible warning from the meter must be followed by a finger-stick test.

Some clinical tests were performed, as reported in ref. [45]: (i) 10 non-diabetic subjects used the meter while their blood glucose was raised from 100 to 300 mg/dl; (ii) 15 non-diabetic subjects used the meter while their blood glucose was clamped at high and normal levels. In another study, presented at ADA2004 Congress, 12 Type 1 diabetic patients underwent a 75 g oral glucose tolerance test, with administration of insulin after 2 h. Moreover, one study was performed to evaluate the effects on the device of motion-induced blood flow variations due to different forearm postures. Fifteen volunteers without diabetes were included, and two devices were fixed at both upper extremities with different fixation techniques (bracelet and tape). Standardized position changes of the upper extremities to induce variations in microcirculation were performed and measured [46].

Test results are the following: (i) 95% of the meter measurements, compared to a gold standard, were in region A + B of Clarke error grid (52% in A, 43% in B); (ii) 99% of the meter measurements, compared to a gold standard, were in region A + B of Clarke error grid (81% in A, 18% in B). In the study on the 12 Type 1 patients, no systematic differences were observed between the meter measurements and the reference values obtained from the fingertip. During slow glucose decrease the meter measurements were in region A of Clarke error grid by 85.1%

(14% in B), whereas during rapid decrease 73.5% were in A and 23.3% in B. With regard to the study on motion effects, some changes in microcirculation were observed in some of the motion actions with consequent influence on the impedance signal. However, these effects were minimized when fixing the device by both bracelet and tape.

The wrist-watch size allows comfortable use. The battery has autonomy up to 24 h. The internal memory can store data for up to 24 h. The meter also has USB connection to a PC for downloading and easy display of glucose readings. A drawback is that, due to differences in the thickness of skin and underlying tissues among individuals, the meter requires a two-point calibration process. Furthermore, some information sources reports that the device does not perform equally in all the individuals.



Figure 43: *Pendra non-invasive glucose meter* <sup>[11]</sup>

### **5.3.3 Meters in process of approval**

#### **5.3.3.1 Aprise™, Glucon Medical Ltd.**

The meter is claimed to be for real-time and continuous blood glucose monitoring, with alarms. It is based on the use of ultrasounds, generated by illuminating the tissue with laser pulses at several selected wavelengths (photoacoustics). Ultrasounds are also used for the localization of the measured volume inside a blood vessel, and for removing the influence of the outer layers of the skin. The Company claims that photoacoustics is superior to traditional optical technologies, as it attains blood glucose measurements directly from inside the blood vessels and allows improved specificity (identification) and sensitivity (detection of level changes) of the glucose measurement.

The Company has performed comparative tests on more than 60 volunteers and diabetic patients in various clinical settings. Blood glucose level was monitored by the Glucon meter continuously (every 10 s) during an OGTT, and sampled every 10–15 min by a reference device. Hypo- and hyperglycemia cycles were also induced, showing accuracy of the device to measure also in the extreme glucose ranges. In a recent note, it is reported that new tests have been performed over 30 diabetic subjects with different protocols to induce glycemic variations: in one protocol dextrose solution and insulin were injected by venous infusion; in the other protocols glucose was administered orally (through a solution or regular meal) followed by diabetic subject's medications.

With regard to the comparative tests on the 60 volunteers, a correlation coefficient  $R$  of 0.94 was found. The mean absolute deviation was 20 mg/dl. In the more recent tests, a mean absolute relative deviation of 19% was found. Moreover the meter is comfortable, since it is wearable like a watch.

#### **5.3.3.2 Glucoband, Calisto Medical Inc.**

The meter has a wrist-watch shape. It is based on the detection of impedance variations in the human tissues due to the application of an electromagnetic field. For each glucose concentration in the body, there is a specific electromagnetic field (called the “glucose signature”) able to produce a resonance phenomenon, which in turn determines the impedance variation. The meter has an internal database of several glucose signatures, each one related to a glucose concentration of a reference solution. Thus, the glucose in the body can be estimated from the specific glucose signature, which determines the resonance phenomenon. The Company calls this approach “Bio-Electromagnetic Resonance (BEMRTM) technology”. The meter is able to provide alarms for both hypo- and hyperglycaemic events, whose thresholds can be set properly for each individual. The measure needs 3–8 min at the most to take place. Measured data can be stored in the internal memory and downloaded to a PC through USB interface.

In a first trial conducted at the City of Angels Hospital in Los Angeles 44 subjects measured their blood glucose level by the Glucoband and by a traditional meter. No details about the studied subjects are provided. In this trial, the correlation coefficient between Glucoband and traditional meter measures was 0.89. In the Clarke error grid 79% of the samples were in region A and 21% in region B. The mean absolute deviation was 19 mg/dl.

The meter is comfortable to be worn. It is powered with batteries lasting 3–6 months. It does not use any disposable material. Calibration occurs automatically in a few seconds before each measurement. The Company emphasizes that the device is absolutely painless and risk-free.

### **5.3.3.3 GlucoTrack™, Integrity Applications Ltd.**

It is a hand-held meter based on ultrasonic measurements to get the glucose level in the blood. To achieve more accuracy and precision, the meter also measures tissue conductivity and heat capacity. The manufacturing company claims that the approach of using three technologies is unique. The meter is made of two units: main unit, containing display, transmitter, receiver and processor, and ‘‘Earring’’ Unit, containing sensors and calibration electronics, to be attached to the earlobe. The meter normally measures spot glucose level, but a continuous mode can be set to perform sequential measurements automatically, thus indicating trends. Extreme values, as set by each user, cause visual and audible alerts. The measured data can be downloaded for further analysis through USB port or IR interface. The meter can be used by Type 1, Type 2, gestational diabetic subjects and by subjects with Impaired Glucose Tolerance. It is claimed that the accuracy of the measurements enable the diabetic patients to decide about the optimal timing to take their medicine. A specific application that is mentioned is related to pediatric incubators, to keep tracking of low glucose level of premature babies, providing alerts when a hypoglycemia episode occurs.

A validation test was performed on 69 subjects, on which 174 measurements were taken globally with the meter and compared to a gold standard. Calibration data of over 3 months old were used up to 7 months old. In this study, the correlation coefficient  $R$  equal to 0.94 was found. The meter is easy to use and allows easy reading thanks to the large LCD screen. Furthermore, the meter supports up to three users, thus making the device more convenient and comfortable in cases of multiple diabetic subjects in the family: the meter does in fact maintain each individual’s data, while each individual has his calibrated earring unit, which can be distinguished thanks to different colours. The meter can work with batteries, and can be easily fastened to the patient’s belt. A disadvantage is that, differently to other non-invasive meters, it requires disposable tapes. The meter requires calibration rarely (less than one per month) differently than many other non-invasive meters requiring calibration every few hours or days. The manufacturer also claims that the technology used is particularly safe for the patient, with no side effects. According to the manufacturer, the next working steps will be final-stage development, miniaturization, completion of clinical trials and regulatory processes.

#### 5.3.3.4 OrSense Ltd.

In this meter, near infrared spectroscopy is complemented with the study of the red band, as well as with a special technique of occlusion and release of the blood vessels in one finger. The Company calls its unique technology red near-infrared (RNIR) occlusion spectroscopy. With this technique a temporary cessation of the tissue's blood flow at the testing site (the finger) is obtained. The Company calls this condition "artificial kinetics". While projecting a multi-wavelength RNIR source, the occlusion and release action causes enhanced aggregation (rouleaux) of red blood cells, which in turn changes the detected RNIR optical signal in relation to the blood glucose, the hemoglobin/hematocrit ratio and oxygen saturation concentration. The optical properties of blood are affected by the concentration of each constituent and the phenomenon is enhanced by occlusion. For each combination of concentrations, there is a specific optic profile ("fingerprint"). By matching the patient's data to a known "fingerprint" an algorithm deduces the concentration of glucose, as well as the other mentioned blood constituents. Occlusion spectroscopy overcomes the problem of the very low signal-to-noise ratio inherent in non-invasive blood measurement. The manufacturer claims that the meter is adequate for both screening and diagnostic purposes, and, more specifically, to monitor post-prandial events, hyperglycemia and hypoglycemia. Measurements are available within 1 min.

A first study was conducted on five men with Type 1 diabetes, which underwent a hyperinsulinemic - hypoglycemic clamp: insulin was infused at a constant rate, and stepped hypoglycemia was achieved by varying glucose infusion. Glucose values ranged from 40 to 270 mg/dl. A second, wider study was performed: 41 subjects were studied (17 females and 24 males, aged 19–82 years, 7 with Type 1 diabetes, 28 with Type 2 diabetes and 6 healthy volunteers). Measurements were performed after a 4-h fasting period for 1 h and hence for other 3 h after a light lunch. Reference blood glucose levels were evaluated every 30 min concurrently with non-invasive readings. Measurements were performed on 10 consecutive workdays. Calibration algorithms were formulated for each subject based on data from the first 2 days of the 10-day trial. In the first study, high degree of correlation ( $R = 0.91$ ) with a gold standard was found, with mean error less than 10%. In the second study, the meter glucose measurements showed again good correlation with the gold standard ( $R = 0.71$ ); the relative absolute difference was 14% and the mean absolute error was 20 mg/dl (10–25 range). Furthermore, the trial indicated that the meter may be used to detect glycemic excursions. The manufacturing company claims that the accuracy level is similar to that of invasive meters used in home care, since they reach a 10% error rate due to handling errors, impure blood intake, etc.

The meter is portable, and very handy primarily due to the infrequent calibration requirements. The meter has an easy-to-read display and an internal memory able to record over 500 entries (blood glucose values, daytime references, etc.). The meter also includes additional features for improved usability, such as trend analysis data and alarm warning on low or high glucose values. It is also claimed that the meter supports telemedicine applications. The manufacturer claims his meter safe, with no hazardous material handling and no risk of contamination for the patient or medical professionals.

#### **5.3.3.5 SpectRx Inc.**

The meter is made mainly of two units: the first unit is a handheld laser, which creates micropores in the external, dead layer of the skin. The micropores have the size of a hair. The interstitial fluid, containing glucose, flows through the micropores and is collected by a patch. Then, it reaches a glucose sensor, which is the second unit. Since the sensor comes in direct contact with the interstitial fluid, a traditional sensor can be used. The meter also includes a transmitter that sends wirelessly the glucose measurements to a handheld display device. The Company calls this methodology “biophotonic technology”

There has been some clinical tests carried out by the manufacturer: (i) 64 adult subjects (29 Type 1 diabetic, 31 Type 2 diabetic, 4 non-diabetic) were studied to know how long the interstitial fluid can be acquired by the micropores; (ii) 30 children, ranging in age from 7 to 18 (19 Type 1 diabetic, 11 non-diabetic) were studied to evaluate interstitial fluid flow rates; (iii) 36 subjects (16 Type 1 diabetic, 9 Type 2 diabetic, 11 non-diabetic) were studied to investigate whether the amount of interstitial fluid harvested is the same for different sites on the body, such as arm or abdomen; (iv) in 20 subjects (all diabetic) the meter measurements were taken for 4–8 h, and compared to finger-stick blood measurements, in order to evaluate the correlation coefficient. A total of 280 paired measurements were taken; (v) in 27 subjects (all diabetic) the meter measurements were taken for 4–8 h, and compared to finger-stick blood measurements, in order to evaluate both correlation coefficient and mean delay time. A total of 738 paired measurements were taken; (vi) in 8 subjects (all diabetic) the meter measurements were taken for overnight periods (8–48 h), and compared to finger-stick blood measurements, in order to evaluate the correlation coefficient. A total of 263 paired measurements were taken; (vii) in 56 subjects (all diabetic) the meter measurements were taken consecutively for 2 days and compared to finger-stick blood measurements, in order to evaluate the correlation coefficient. A total of 1167 evaluable paired measurements were obtained; (viii) in 252 (187 diabetic, 65 non-diabetic) subjects the meter measurements were taken consecutively for 2 days and compared to

blood measurements through a commercial blood glucose system, in order to evaluate the correlation coefficient. A total of 4059 evaluable paired measurements were obtained.

Only some information on results of the above-mentioned tests are reported: (i) interstitial fluid can be acquired for up to 96 h; (ii) interstitial fluid flow rates are slightly higher for children than for adults, and hence system may be adaptable for use in children; (iii) the amount of interstitial fluid harvested is nearly the same for the different body sites; (iv) the correlation coefficient  $R$  was 0.901; (v) the correlation coefficient  $R$  was 0.894, and the mean time delay between interstitial fluid and blood measurements was 11.5 min; (vi) the correlation coefficient  $R$  was 0.898; (vii) the correlation coefficient  $R$  was 0.875, with 97.7% of points within A + B regions of Clarke error grid; (viii) the correlation coefficient  $R$  was 0.946, with 99.1% of points within A + B regions of Clarke error grid

The manufacturer claims that the system was designed to be comfortable to wear.

#### **5.3.3.6 SugarTrac™, LifeTrac Systems Inc.**

The meter has the size of a mobile phone. A low energy beam of near IR light is transmitted through the earlobe thanks to an engaged earpiece, thus allowing measurement of glucose in the patient's blood. The earpiece has to remain on the ear for less than 1 min. The meter is not intended for continuous use, but it allows an unlimited number of readings. A 5 min wait between readings is recommended. The meter includes light sources having a wavelength of 940 nm to illuminate the tissue, and receptors for receiving light and generating a transmission signal. The light sources are LED pulsed at 1 kHz for a 1 ms pulse width. A signal analyser, which includes a trained neural network, determines the glucose concentration in the blood of the subject.

A clinical trial at Brigham & Women's Hospital, Biddeford, ME, seems to have occurred, this study carried out by the manufacturer showed a correlation coefficient  $R$  of 0.88 with a reference glucose meter.

Good usability is allowed by its compact size and by the comfortable power supply simply based on a standard 9 V battery. The Company claims that the transmitting diode is only active for 5 s, and it is impossible to look directly into with an opening of only 0.531 of an inch. In any case, near infrared light is not damaging a human eye. Furthermore, there is no contact to the skin possible from the photo diode (transmitter) or the photo pickup (receptor), due to the fact that the transmitter and receptor are embedded into nylon plastic, prohibiting contact with any metal parts. The only part a user will come into contact with will be the glass domes of the transmitter and receptor, and the plastic surrounding them. In any case, the voltage to the

earpiece is only 0.17 V. The Company also claims that the earpiece is not damaging the earlobe: the maximum opening of the earpiece is such that the pressure exerted is comparable to that of a clip-on earring.

#### **5.3.3.7 Symphony™, Sontra Medical Corporation**

The meter is made essentially of two units: an ultrasonic device, coupled with the skin through an aqueous medium, which increases skin permeation, and a glucose biosensor, which measure glucose in the interstitial fluid reaching the sensor through the micro-pathways generated on the skin [49]. From the biosensor, some data are transmitted through wireless technology to a glucose meter that eventually provides and displays the glucose reading.

In the study [49], 10 patients with Type 1 or 2 diabetes were studied for a period of 8 h. Blood glucose measurements were taken every 20 min, whereas the glucose sensors placed over the treated skin sites (two per person) provided readings every 5 s. In this study, a correlation coefficient  $R$  of 0.84 is reported, with 95% of the glucose measurement pairs in the A + B region of the Clarke error grid. In a second study with similar conditions, a correlation coefficient  $R$  of 0.90 is indicated, but no further details are provided.

It is claimed that the application of the ultrasonic device for 15 s is enough to make the skin permeable for several hours. The technology is absolutely painless. In the study [49], it is reported that no patient suffered pain or skin irritation during the study.

### **5.3.4 Meters poorly described**

In this section, the devices included match the inclusion criteria of non-invasive blood glucose measurement devices, but with very poor information on them. In particular, there are no concrete results of tests on these devices.

#### **5.3.4.1 Dream Beam, Futrex Medical Instrumentation Inc.**

The meter measures the blood glucose in a finger. Futrex claimed to have successfully tested the meter at Mount Sinai Medical Center in New York in May of 1991. However, the Company was asked to repeat the Mount Sinai study at hospitals in the Washington/Baltimore area in 1992, and it seems that these tests, which were analysed by Eli Lilly, failed to show that Futrex's meter could correctly ascertain blood glucose levels. In 1998 new tests were performed



in Chile, especially to study the glycemic oscillations in children with insulin-dependent diabetes, and to determine the meal order effects on post-prandial hyperglycemia.

Good usability is allowed by its compact dimensions, which make it portable, and by the use of batteries to power the meter. However, it requires individual calibration.

#### **5.3.4.2 GluCall, KMH Co. Ltd.**

The meter allows continuous glucose measurements in the interstitial fluid. It is worn like a wrist-watch and it is used with a single-use disposable sensor, which is attached to the backside of the meter case and is in contact with the skin. Each sensor provides up to three glucose measurements in 1 h for 6 h, giving up to 18 readings per use. To obtain the measurement a very low level of direct current is applied across the skin to get the interstitial fluid. Thanks to reverse iontophoresis the glucose in the interstitial fluid is extracted and collected into the two hydrogel disks in the sensor. Afterwards the extracted glucose reacts with the glucose oxidase (GOD) enzyme immobilized in the hydrogel disks to form hydrogen peroxide ( $H_2O_2$ ). Hydrogen peroxide is oxidized on a platinum electrode to produce electric currents. These signals are translated into an equivalent blood glucose level by a data conversion algorithm. The measuring range goes from 50 to 600 mg/dl. The meter has alarms for high and low glucose level, as well as for rapid changes.

The meter is comfortable to be used since it can be worn like a watch. It also has a large LCD display. Furthermore, the meter internal memory can store up to 8000 readings and special events can be marked. The readings can also be downloaded to a PC where a software tool allows easy data display and management. One drawback of the meter is that before performing the glucose measurements it needs about 1 h of warming period, and afterwards it has to be calibrated through a traditional blood glucose meter.

#### **5.3.4.3 GluControl GC3001, ArithMed GmbH**

The meter monitors blood sugars in the 50–400 mg/dl range. Blood glucose values, time and date are automatically stored in the meter memory. It measures 17.6 cm - 11.6 cm - 6 cm, with a weight of 500 g.

It has been declared that clinical trials have been conducted in Germany, Japan and Korea, but no details are provided. The meter does not require additional materials. It works with batteries.

#### **5.3.4.4 Hitachi Ltd.**

Hitachi claims the development of a non-invasive blood sugar monitoring device based on the use of special sensors to detect physiological parameters related to human metabolism. In fact, extensive Company studies showed that thermal energy generated by metabolic reactions in the human body reflects a balance between blood sugar levels and local oxygen supply. Therefore, it is possible to determine the level of blood sugar by measuring the thermal energy generated by the metabolic reactions, together with other parameters, such as the level of oxygen saturation of hemoglobin and the blood flow. The meter uses sensors to measure various temperatures and light characteristics in the fingertip. The Company has in fact developed a complex sensor, which contains a contact thermometer, a radiation thermometer, and a multi-wavelength reflective photometer. To execute the test, the patient places a finger on the meter for 10 s and repeats the action 2 min later. The meter takes a further 2 min to display the results.

All the device units are integrated in a unique sensor, and hence the meter has a compact size that makes it easily portable and adequate for home use.

#### **5.3.4.5 Sysmex Corporation**

The meter has a wrist-watch format. The meter is based on a new fluid extraction technology developed by the Company, which is able to provide stable interstitial fluid samples, and on a glucose biosensor chip (developed by another Company, Toshiba) able to measure the little amounts of glucose in the interstitial fluid samples.

The compact format should allow a comfortable use.

#### **5.3.4.6 TouchTrak Pro 200, Samsung Fine Chemicals Co. Ltd.**

The meter is a hand-held device, which measures blood glucose levels. Some sources of information indicate it as the first non-invasive glucose meter commercialized in the world (commercial production seems to have started in 1998, at a price around US\$ 30,000). Another meter, cheaper (around US\$ 3000) and hence for personal, domiciliary use (TouchTrak HC 300) should have been developed and put on the market around the end of 1999. However, it was not possible to find further information on this second meter. It seems that in 1999 the Company was performing clinical trials both in Korea and in the US, but no further details were found.

## 6. Conclusions

In conclusion, we can say that there are many alternative possible methods for the non-invasive measurement of the blood glucose levels. The correlation values of the devices discussed above, based of non-invasive principles, with commonly used devices, based on accurate invasive methods are given. The sensitivity and specificity of these devices are also discussed along with the limitations of each device based on its non-invasive operating principle.

Amongst all the devices, the most unique one is the GlucoTrack<sup>TM</sup>, Integrity Applications Ltd., which is currently in process of approval, due to its novel approach of integrating 3 different technologies, tissue conductivity, heat capacity and ultrasound technology, into one device. This, I believe is the novel approach and Integrity Applications Ltd. Deserves the credit for it for suggesting it. The reason for my interest in this is simple. Since each working principle has its own drawbacks, one device should employ more than one working principle via integration of various non-invasive measurement techniques in order to make up for the drawbacks of each other. For this to become possible further advancements in miniaturization of each of these methods to be integrated is necessary. I believe, this integration method will finally enable the engineers to develop non-invasive devices for the measurement of blood glucose as accurately as invasive and minimally invasive methods, maybe as well as the measurements of other vital biochemical in later stages.

## **APPENDIX I: ACRONYMS**

DRI: Daily Relative Intake

AD: Antidiuretic Hormone

RBC: Red Blood Cell

ALT: Alanine Transaminase

AST: Aspartate Transaminase

OGGT: Oral Glucose Tolerance Test

## APPENDIX II: REFERENCES

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