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TRACEABILITY OF RESULTS AND COMPARISON OF ROUTINE METHODS IN THE MEDICAL LABORATORY

Master`s Thesis

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Abbreviations

BD Becton Dickinson

CLSI Clinical and Laboratory Standards Institute

CSF Cerebrospinal fluid

GC-MS Gas chromatography mass spectrometry

IFCC WG-SEPOCT International Federation of Clinical Chemistry Workgroup Selective Electrode and Point Of Care Testing

IRP International reference preparation

IS International Standard

IV intravenous

MI Myocardial infarction

NIBCS National Institute for Biological Standards and Control

NIST National Institute for Standards and Technology

POCT Point of care test

RIA Radioimmunoassay

SRM Standard Reference Materials

Introduction

The value of laboratory medicine is realized through its many roles in patient care. These include screening of asymptomatic individuals to identify risk for developing disease, detecting disease at the earliest stages before symptoms occur, selecting safe and effective treatments, planning disease management strategies, estimating treatment response throughout the course of care, identifying threats to patient safety and public health, such as hospital-acquired infections, protecting the blood supply and transplant recipients from harmful pathogens, and drugs of abuse testing to support clinical care and assure public safety.

Laboratory medicine also is important to clinical guidelines. As described in [1] a search of clinical practice guidelines across 23 main condition/disease categories found that 37 % focused on or involved laboratory tests. New testing techniques tend to have lower detection limits (LoD) and higher specificity, enabling clinicians to detect, diagnose, and manage diseases more effectively.
Laboratory investigations have a major effect on clinical decisions, providing physicians, nurses, and other healthcare providers with information that aids in the prevention, diagnosis, treatment, and management of diseases.

Tartu University Hospital is the largest provider of medical care in Estonia. The number of outpatient visits per year is up to 420 000 and about 13 % of visits are made to emergency medicine units. The number of inpatients is around 44 000 [2].The United Laboratories is one of the 25 medical (17 clinics and 8 medical services) departments of Tartu University Hospital. More than 1 500 different laboratory tests are available for clinical use in the United Laboratory of Tartu University Hospital. Laboratory services account for only 1,8 % of total Hospital health care expenditures; but up to 70 percent of health care decisions are based on the results of those tests, making medical laboratories a vital part of the health care system [2].

The aim of the present work is to assess the performance of laboratory and POCT methods for different analyses and tests of clinical importance, to analyze the bias between two methods in a number of pairs and to develop a system for results recalculation in order to remove the bias and establish traceability

The report consists of two main parts. The first, theoretical part gives a short overview of the laboratory methods, standardization, harmonization and traceability in laboratory medicine

and also routine methods that are used in medical laboratories and clinical departments for carrying out laboratory tests. The second part focuses on comparison of different methods for glucose determination and methods for measurements of concentrations of different hormones.

1. Definitions [3]

quantity

property of a phenomenon body, or substance, where the property has a magnitude that can be expressed as a number and a reference

measurement

process of experimentally obtaining one or more quantity values that can reasonably be attributed to a quantity

measuring system

set of one or more measuring instruments and often other devices, including any reagent and supply, assembled and adapted to give information used to generate measured quantity values within specified intervals for quantities of specified kinds

measuring instrument

device used for making measurements, alone or in conjunction with one or more supplementary devices

measurand

quantity intended to be measured

reference measurement procedure

measurement procedure accepted as providing measurement results fit for their intended use in assessing measurement trueness of measured quantity values obtained from other measurement procedures for quantities of the same kind, in calibration, or in characterizing reference materials

primary reference measurement procedure

reference measurement procedure used to obtain a measurement result without relation to a measurement standard for a quantity of the same kind

calibration

operation that, under specified conditions, in a first step, establishes a relation between the quantity values with measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication

calibration hierarchy

sequence of calibrations from a reference to the final measuring system, where the outcome of each calibration depends on the outcome of the previous calibration

metrological traceability

property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty

metrological traceability to a measurement unit

metrological traceability where the reference is the definition of a measurement unit through its practical realization

NOTE The expression “traceability to the SI” means ‘metrological traceability to a measurement unit of the International System of Units’.

measuring interval

set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental measurement uncertainty, under defined conditions

measurement standard

realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference primary standard measurement standard established using a primary reference measurement procedure, or created as an artifact, chosen by convention

secondary measurement standard

measurement standard established through calibration with respect to a primary measurement standard for a quantity of the same kind

reference material, RM

material, sufficiently homogeneous and stable with reference to specified properties, which has been established to be fit for its intended use in measurement or in examination of nominal properties

certified reference material, CRM

reference material, accompanied by documentation issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceabilities, using valid procedures

commutability of a reference material

property of a reference material, demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specified materials

2. Literature overview

2.1 Traceability

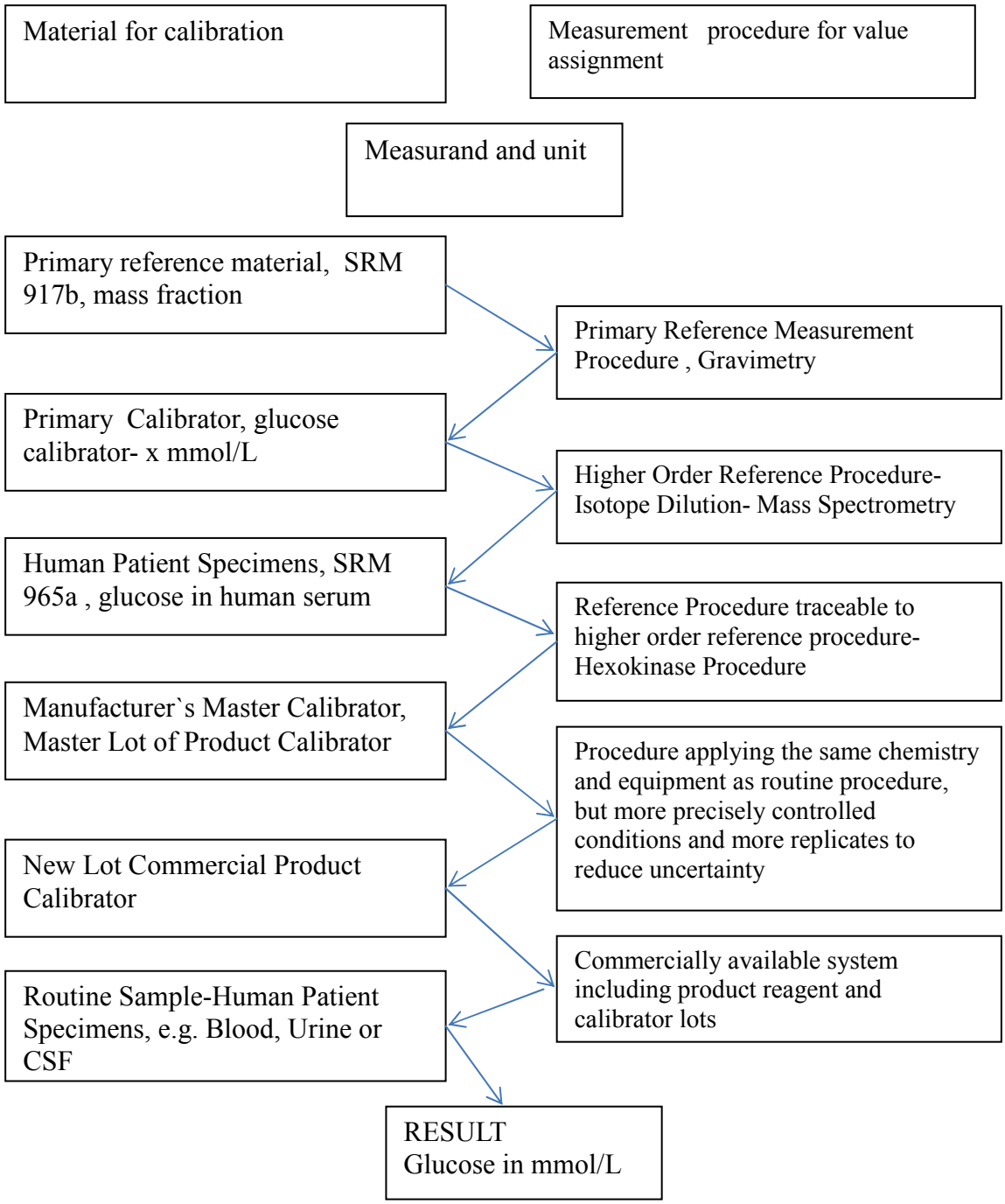
The term “traceability” originates in the metrological community, where it was first defined in 1993 in the predecessor of the current International Vocabulary of General and Basic Terms in Metrology [3]. In the same year, the Cooperation on International Traceability in Analytical Chemistry was formed to encourage the broad realization of traceability in analytical chemistry [4]. The implementation of the European Union Directive on in vitro diagnostic devices became mandatory and has had a worldwide effect on instruments and reagents and thus clinical laboratory measurements.

Traceability is realized by relating a measurement result to a stated reference through an unbroken chain of calibrations [3]. Stated references may range from a corporate standard to a certified primary reference material that embodies a unit of the Systéme International (SI). These reference materials must have certain well defined characteristics, such as homogeneity and stability, as described in the ISO 17511 standard [5]. Reference measurement procedures must be characterized, validated and documented according to specifications outlined in documents such as ISO 15193 [6] or those created by the Join Committee for Traceability in Laboratory Medicine (JCTLM) [7] .

The current list by JCTLM includes more than 200 certified calibrators (CRM) for about 130 biomarkers. It also lists more than 125 reference measurement procedures and it includes recognized reference laboratories for specified quantities [8].

Items on the list have been reviewed and internationally agreed to be consistent with criteria described in the standard ISO 15193 [6]. The unbroken chain of calibrations applies a hierarchical order to materials and measurement procedures, as diagrammed in Figure 1 [9].

Figure 1. *Traceability Chain for the Calibrators, on the example of Glucose determination.*



The traceability chain for the concentration of glucose in body fluid to the SI concentration units expressed as amount-of-substance concentration in millimoles per liter (mmol/L) starts with the definition of the measurand as "glucose in blood, serum, urine, CSF; amount-of-substance concentration equal to X mmol/L". The unit is embodied in the Standard Reference Material 917b from the National Institute for Standards and Technology (NIST, USA). This crystalline material consists of chemically purified D-glucose (dextrose) and it is used to prepare the primary calibrator. This calibrator is suitable only for calibrating the secondary reference procedure, the isotope dilution – mass spectrometry measurement procedure, which

is used to measure glucose concentrations in a panel of native materials and it is used to prepare the secondary calibrator – SRM 965a glucose in frozen human serum. Those sera are used for calibrating the manufacturer's master procedure by a method comparison study. The traceability chain shown in Figure 1 illustrates a scenario in which all the necessary components are available to establish traceability to the SI. Although establishing traceability to the SI is the eventual goal for all measurement procedures, this is currently not possible for all analytical determinations in the clinical laboratory.

2.2 Traceability categories

To account for situations, where traceability to the SI cannot be established alternative traceability chains have been defined by ISO 17511 [5] as presented in Table 1.

Table1. *Traceability categories from ISO 17511 [5].*

Category	Reference measurement procedure	Primary (pure substance) reference material	Secondary (value assigned reference material)*	Examples
1	Yes	Yes	Possible	Electrolytes, glucose, steroid hormones, some thyroid hormones, and drugs
2	Yes	No	Possible	Enzymes, hemoglobin
3	Yes	No	No	Hemostatic factors
4	No	No	Yes	Proteins, tumor markers, HIV
5	No	No	No	Proteins, viruses

*More than one secondary reference material, with potentially different properties, may be available for the same measurand.

The five traceability categories defined in this standard are [10]:

1. Measurement results are traceable to an SI unit. The calibration process follows, in principle, the procedure diagrammed in Figure 1 and further explained in detail below.

In this scenario, chemical and physical properties of the analyte are known, and primary reference measurement procedures and calibrators are available.

2. Measurement results are traceable to an international reference measurement procedure (which is not primary) and to international conventional calibrator(s) without metrological traceability to the SI. In this scenario, reference measurement procedures and calibrators are defined by convention or consensus.
3. Measurement results are traceable to an international conventional reference measurement procedure (which is not primary), but there is no international conventional calibrator and no metrological traceability to the SI. In this scenario, the measurement procedure is defined by convention or consensus and no calibrator exists.
4. Measurement results have traceability to an international conventional calibrator (which is not primary), but there is no international conventional reference procedure and no metrological traceability to the SI. In this scenario, a reference material is defined by convention or consensus, and values are assigned to this material in arbitrary units, such as International Units by WHO standards.
5. Measurements have traceability to a manufacturer's selected measurement procedure but there is neither an international conventional reference procedure nor an international conventional calibrator, and there is no metrological traceability to the SI. This scenario occurs when new biomarkers are identified and developed by research laboratories.

The assay manufacturer is responsible for establishing and documenting metrological traceability for commercially available methods and calibrators. Individual clinical laboratories that use these commercial methods do not need to demonstrate traceability as long as the manufacturer's instructions for use are followed.

2.3 Traceability data of immunological tests in the United Laboratories of Tartu University Hospital

Table 2 presents traceability data of immunological tests by two manufacturers that are used at the United Laboratories. One of them (Immulite 2000) was used in the laboratory during 2006-2011 and the other (Cobas 6000) from 2012 to present.

***Table 2**. Traceability for immunological tests used in the United Laboratories*

Quantity	Immulite 2000, Siemens [11]	Cobas 6000, Roche [12]
Adrenocorticotropic hormone	internal standard	gravimetrically produced calibrator
Alpha-fetoprotein	WHO 1st IS 72/225	WHO 1st IS 72/225
Beta-2-microglobuline	internal standard	WHO standard
Cancer Antigen 125	no information	RIA
Cancer Antigen 15-5	no information	RIA
Cancer antigen 19-9	no information	enzymun test
Carcinoembryonic antigen	no information	1st IRP WHO 73/601
C-peptide	WHO 1st IRP 84/510	WHO 1st IRP 84/510
Dehydroepiandrosterone sulfate	no information	gravimetrically produced calibrator
Ferritin	WHO 2nd IS 80/578	1st 80/602, 2nd IS 80/578, 3rd IS 94/572
Folic acid	internal standard	Folate II gen
Follicle stimulating hormone	WHO 2nd IRP 78/549	WHO 2nd IRP 78/549
Human chorionic gonadotropin	1st IRP 75/551	4st IRP 75/589
Insulin	WHO NIBSC 1st IRP 66/304	WHO NIBSC 1st IRP 66/304
Cortisol	internal standard	IDMS
Luteinizing hormone	WHO 1st IRP 68/40 2nd IS 80/552	2nd NIBSC 80/552
Progesterone	internal standard	ID-GC/MS
Prolactin	3rd IS 84/500	3rd IRP WHO 84/500
Prostate specific antigen	internal standard	WHO 96/670 (Stanford ref.standard)
Free prostate specific antigen	WHO NIBSC 1st 96/668	WHO NIBSC 1st 96/668
Parathyroid hormone	internal standard	RIA
Sexual hormone binding globulin	no information	1st NIBSC 95/560

Triiodothyronine	no information	IRMM-469
Free triiodothyronine	internal standard	equilibrium dialysis
Thyroxine	internal standard	ID-GC/MS
Free thyroxine	internal standard	equilibrium dialysis
Testosterone	internal standard	ID-GC/MS
Anti-thyroglobulin	WHO 1st IRP 65/93	NIBSC 65/93
Anti-thyroid-peroxidase	WHO 1st IRP 66/387	NIBSC 66/387
Thyroid stimulating hormone	2nd IRP WHO 80/558	2nd IRP WHO 80/558
Thyroglobulin	CRM 457	CRM 457
Vitamin B_{12}	internal standard	internal standard
Estradiol	internal standard	ID-GC/MS
Immunoglobulin E	2nd IRP WHO 75/502	2nd IRP WHO 75/502

2.4 Traceability challenges

2.4.1 Commutability of reference materials

Commutability is a property of a reference material [3], demonstrated by the closeness of agreement between the relation among the measurement results for a stated quantity in this material, obtained according to two given measurement procedures, and the relation obtained among the measurement results for other specific materials.
A commutable reference material has the same signal to concentration ratio as the native samples and the same performance with the measurement procedures in the traceability chain. Differences in analytical response between the reference material and the clinical samples will introduce a bias in the result. Commutability is especially important for reference materials that are used to measure the quantity directly in the sample matrix (e.g. serum, blood, urine) without any prior isolation or purification steps [13].

2.4.2 Free and complex molecules

The definition of the measurement system (e.g. blood, plasma, serum) may seem obvious for many quantities. In some cases, however, transferring the theoretical concept into the practice of measurement may be problematic. Consider, for example, serum water (relevant for free

hormones, e.g. free T3, T4, and PSA and plasma glucose concentrations). Many components in serum are bound to proteins or form complexes with other components. Therefore, their concentrations in serum water differ from their concentrations in serum. Currently, no measurement methods of the "true" concentrations of such components in serum water by directly measuring serum samples are available. Serum water must be separated from serum by ultra filtration or dialysis.These processes break the traceability chain. An example of the effects of such constraints is the required definition of the measurand for free thyroxine (fT4) as "equilibrium-dialysate from serum prepared under defined conditions: thyroxine (free) expressed in pmol/L [10].

2.4.3 Different molecular forms

Defining the quantity to be measured may pose challenges. Parathyroid hormone (PTH) assays able to distinguish between the full-length PTH (PTH1-84) and N-terminally truncated PTH (PTH 1-34) are used to increase the significance in the acute diagnosis of endocrine and osteological diseases. Almond et al. [14] studied the performance of six different current commercial PTH immunoassays and found an up to 4.2 fold difference between the lowest and highest results in the same patient sample. It is not evident from the report which calibrators were used and their traceability.

2.4.4 Different binding sites in immunoassays

Immunoassays of different origins may be specific for certain antigen-antibody binding sites (also called epitopes) but still be intended for measuring the same quantity, the measurand, which is of clinical or physiological interest. The assay thus measures the concentration of a particular component and it would be misleading to define the measurand (the clinically important quantity) by the epitope used [10].
Data on captured (C) and detection (D) troponin antibodies, recognized aminoacids and epitope tagging (Tag) are provided in Table 3. Troponin is a "gold marker" in the diagnosis, prognosis and strategy selection for acute coronary syndrome.

Table 3*.Analytical characteristics of commercial cardiac troponin assays public and private laboratories in Estonia. [15-16].*

Hospital	Company/platform (s) /assay	Troponin T TnT	Epitopes to recognized by	Detection Antibody	cut-off for MI

		Troponin I TnI	Antibodies	Tag	
SA Pärnu Haigla	Abbott/Architect ci4100	TnI	C:87-91, 24-40; D:41-49	Acridinium	28 ng/L
SA Läänemaa Haigla	Siemens/ADVIA Centaur CP	TnI	C:41-49, 87-91; D:27-43	Acridinium	40 ng/L
SA Ida-Viru Keskhaigla	Siemens/Immulite	TnI	C:87-91; D:27-40	Alkaline phospatase	300 ng/L
Quattromed HTI Laborid OÜ	Siemens/ADVIA Centaur XP	TnI	C:41-49, 87-91; D:27-43	Acridinium	40 ng/L
AS Lääne-Tallinna Keskhaigla	Abbott/Architect ci8200	TnI	C:87-91, 24-40; D:41-49	Acridinium	28 ng/L
SA Viljandi Haigla	Roche/Elecsys 2010, Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Tartu Ülikooli Kliinikum	Roche/ Cobas e 601	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Rapla Maakonnahaigla	Roche/Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Põhja-Eesti Regionaalhaigla	Roche/ Cobas e 601	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Narva Haigla	Roche/Elecsys 2010, Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Jõgeva Haigla	Roche/Elecsys 2010	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
SA Hiiumaa Haigla	Roche/Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14ng/L
Kuressaare Haigla SA	Roche/Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
AS Valga Haigla	Roche/Elecsys 2010, Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
AS Rakvere	Roche/ Cobas h	TnT	C:125-131;	Ruthenium	below LoD

Haigla	232		D:136-147		
AS Põlva Haigla	Roche/Elecsys 2010	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
AS Lõuna-Eesti Haigla	Roche/Elecsys 2010	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L
AS Järvamaa Haigla	Roche/Cobas h 232	TnT	C:125-131; D:136-147	Ruthenium	below LoD
AS Ida-Tallinna Keskhaigla	Roche/ Cobas e 601, Roche/Cobas e 411	TnT	C:125-131; D:136-147	Ruthenium	14 ng/L

Clinical decision limit (also called cut-off) is the troponin concentration at the 99th percentile, measured in a healthy reference population, and with assay imprecision of 10% or less. 5of 19 hospitals used troponin I assays and clinical decision limits depends from manufacture and /or platform : 28, 40 and 300 ng/L. Other hospitals used troponin T assay, two of them used POCT- devises. The clinical decision limit of troponin T is14 ng/L, except Cobas h 232, because imprecision of POCT devise at cut-off level is > 10% and closed to limit of detection.

2.4.5 Auto antibodies

Auto antibodies against cardiac troponins interfere with the measurement of cardiac troponin I by immunoassays for the diagnosis of myocardial infarction. cTnAAbs have been found in high proportion (5-20 %) of individuals with and without cardiac diseases. Cardiac troponin-specific autoimmune response leading to autoantibody formation can be triggered by any release of cardiac proteins following myocardial injury, for example, after inflammation, ischemia or cardiotoxic treatments [17].

2.4.6 WHO materials preparation

Many immunoassays are calibrated using the WHO materials. WHO has developed the International Unit (IU) concept according state-of-art purification and identification techniques, and its function, or potency, is tested by response of a biological system [10]. The IU is then assigned by convention (e.g., 1 IU is assigned to 1 mg preparation). Once defined, the IU is passed to all further International Standard preparations by use of traceability

protocol. Many immunoassays are calibrated with such materials; however, the traceability to the WHO unit is broken by the non-commutability of the WHO materials with native patient samples. This problem is reflected by differences in results among various methods that are nominally calibrated with the same WHO material (C-peptide, insulin) [10].

2.5 Glucose

Glucose is a carbohydrate ($C_6H_{12}O_6$) and one of the major sources of energy for organ function [18].
Measurements of glucose concentrations are fundamental in the diagnosis of diabetes, to monitor critically ill patients on glycemic control protocols and to estimate hypoglycemia in the newborn. Blood glucose concentrations may be measured in the laboratory and in point-of-care environments using special glucose meters.

2.5.1 Glucose measurement procedures in Tartu University Hospital

Table 4 presents the glucose measurement procedures used in Tartu University Hospital together with their sources of traceability.

Table 4.Glucose measurement methods traceability [19-23] in Tartu University Hospital 2010-2013

	United laboratory		Point of care test (POCT)		
	Cobas 501 Roche	ABL 800 Radiometer	Stat Strip Nova	Glucocard Arkray (before 2012)	HemoCue (before 2012)
Sample for measurement	serum, plasma	blood	blood	blood	blood
Calibration of method	NIST SRM 965a	NIST SRM 917a	comparison with method for plasma	comparison with method for plasma	comparison with method for plasma

POCT glucose meters have been shown less precise than laboratory-based instrumentation, and results may differ significantly from laboratory methods [24].

2.5.2 POCT advantages

A major advantage of POCT is that it provides much faster access to test results, allowing for more rapid clinical decision making and more appropriate treatments and intervention. In addition, POCT can help minimize time-depending changes in labile analytes such as glucose, for instance occurring during sample transport to the laboratory. Finally, POCT methods usually require much smaller sample volumes (1-2 µL) than those needed for testing in the laboratory [25].

2.5.3 Glucose in blood

In human blood, glucose, like water, is distributed between erythrocytes and plasma (Figure2). The molality of glucose is the same throughout the sample, but the concentration is higher in plasma than erythrocytes.

Figure 2. Glucose concentration in plasma, calculated from B-Glucose with correction for the hematocrit

Blood (Hematocrit h %)	Percentage water	Total percentage water in plasma & cells, resp.	Total percentage water in blood
Plasma (100-h) %	93 %	(100-h) *x* 93/100	(93 *x* (100-h)+71 *x* h)/100
Cells h %	71 %	h *x* 71/100	

Water content expressed as percent volume [26].
With a hematocrit of 43 % and water content for the fraction "Cells" of 71 %, the total water content of the cells will be 31 % of total volume (whole blood). In plasma, 93 % will be water, thus giving a water content of 53 % for the plasma portion of whole blood. The total water content of blood then is 84 % (53 %+31 %). For a hematocrit of 43% the plasma/whole blood ratio of water content is 0,93/0,84=1,11, which explains the 11 % higher glucose values in plasma compared to whole blood and a correction factor of 1,11 [26].
The IFCC-SD, WG-SEPOCT recommends reporting the concentration of glucose in plasma, irrespective of sample type or measurement technique. If the measurement procedure includes a hemolysis of the blood then a constant factor is used to convert the B-Glucose concentration to P-glucose. Most test strips use the porous layer technique to separate the blood cells and lipids thereby measuring glucose concentrations in plasma/plasma-like fluids. In theory, the

only systems that should not be affected by hematocrit are instruments using direct-reading electrodes. [27].

Critically ill patients may have a very low hematocrit (e.g. 25 %) and the plasma/whole blood ratio of water content is 0,93/0,88=1,06, which should be used to estimate the glucose concentration in plasma from a result obtained in whole blood.

Many institutions use tight glycemic control (TGC) protocols in their intensive care units. TGC protocols became standard of care after initial, very promising, studies demonstrating that it improved patient outcomes and reduced mortality [28].

Table 5. *Tight Glycemic Control protocol in ICU.*

Glucose in blood, mmol/L	Insulin-dosing category	Insulin infusion rate IU/h			
hourly basis		algorithm 1	algorithm 2	algorithm 3	algorithm 4
<4	1	0	0	0	0
4-6	2	0,2	0,5	1	1,5
6-7	3	0,5	1	2	3
7-8	4	1	1,5	3	5
8-10	5	1,5	2	4	7
10-12	6	2	3	5	9
12-13	7	2	4	6	12
13-15	8	3	5	8	16
15-17	9	3	6	10	20
17-18	10	4	7	12	24
18-20	11	4	8	14	28
>20	12	6	12	16	28

TGC protocol consist of placing postoperative and critical ill patients on a continuous intravenous insulin infusion, checking their blood glucose concentration on an hourly basis (or other schedule), and giving a bolus of insulin and/or changing the infusion rate of insulin based on the glucose concentration, with a goal to maintain glucose between 4,4-6,7 mmol/L.

The most critical errors to prevent are those that are likely lead to hypoglycemia, which may be lethal. An incident and constant observation from many studies is that severe

hypoglycemia (glucose < 2,2 mmol/L) in a population of patients by logistic regression is associated with a sixfold increase in death [29]. Hyperglycemia is toxic and may lead to coma, which, if not treated, is life-threatening. Analysis of 259 040 ICU patients showed that hyperglycemia (glucose concentration >6,1 mmol/L) was associated with mortality independent of illness severity, type of ICU or lengths of stay [30]. Randomized controlled trials [31] showed an increased frequency of hypoglycemia after tight glycemic control to reduce long-term complications; there is an increase of hypoglycemic episodes in critically ill patients when strict glycemic control is established.

Neonates may have a very high hematocrit (70%) and the plasma/whole blood ratio of water content is 0,93/0,78=1,20, which reflects the 20 % higher glucose values on plasma compared to whole blood. Hypoglycemia in term infants has been defined as a blood glucose concentrations less 2,2 mmol/L. The overall incidence of hypoglycemia has been estimated at 1 to 5 per 1,000 live births. The goals in treating the infant who has hypoglycemia are to normalize blood glucose concentration as quickly as possible by enteral or IV dextrose water solution [19]. It is therefore important to have an accurate measurement procedure available in neonatal care.

2.5.4 Glucose methods specificity

Hexokinase and glucose oxidase measurement methods are specific for glucose. Glucose dehydrogenase pyrroloquinolinequinone (GDH-PQQ) or glucose-dye-oxidoreductase (GDO) methods may result in falsely elevated blood glucose reading in patients using EXTRANEAL (icodextrin) due to maltose interference. EXTRANEAL is used for dialysis of patients. A blood glucose reading with these methods in these patients may mask true hypoglycemia. A falsely elevated glucose could cause a patient to be given more insulin than needed. Both situations can lead to life-threatening events, including loss of consciousness, coma, permanent neurological damage or death [31].

Table 6.*Glucose methods specificity in Tartu University Hospital*

	United laboratory		Clinical departments (POCT)		
	Cobas 501 Roche	ABL 800 Radiometer	Stat Strip Nova	Glucocard (before 2012)	HemoCue (before 2012)
method	hexokinase	glucose oxidase	glucose oxidase	glucose dehydrogenase	glucose dehydrogenase

specificity	yes	yes	yes	no	no

In Estonia, as well as in other European countries, medical equipment has been listed in national registers, but it does not have information for measurement method [32].

2.6 Hormones and tumor markers

Follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol affect the reproductive organs of both the female and the male. Abnormal spermatogenesis is often associated with altered serum FSH and LH levels.

Prostate-specific antigen (PSA) is a glycoprotein that is produced by the prostate gland. PSA exists in serum in multiple forms: complexed to alpha-1-anti-chymotrypsin (PSA-ACT complex), unbound (free PSA), and enveloped by alpha-2-macroglobulin (not detected by immunoassays). Higher total PSA levels and lower percentages of free PSA are associated with higher risks of prostate cancer.

Alfa-fetoprotein (AFP) is a glycoprotein, synthesized by the embryonic yolk sac, non-differentiated liver cells, and the gastro-intestinal tract. AFP levels are measured in pregnancy to screen for open neural tube defects-incidence, Down syndrome and in adults to detect liver cancer and germ cell tumors.

3. Experimental

3.1 Study of performance of glucose meters

The first study was performed based on the data obtained during a one month period from the Intensive Care Unit of the Anesthesiology and the Intensive Care Clinic (ICU). No additional blood drawing or finger stick samples were used. The correlation analysis was performed by analyzing the data of 41 samples using the Nova Stat Strip glucose meter and the ABL 825 blood gas analyzer (Radiometer). The second study was performed using 74 samples during a two week period from the Neonatal Care Unit of Women Clinic (NCU). Method correlation analysis was performed by analyzing the data from the 74 samples measured in parallel on the Stat Strip glucose meter and on the HemoCue glucose meter. Ten samples from the laboratory were measured by blood gas analyzer ABL 825 and two glucose meters (Stat Strip and HemoCue).

3.1.1 Instrumentation

Three blood glucose strip-meter systems suitable for POCT representing different strip technologies were evaluated in this study: Stat Strip (NOVA Biomedical, Waltham, MA), Glucocard X (Arkay Factory, Japan) and HemoCue AB (HemoCue Ltd). Glucocard was used in ICU and HemoCue in NCU from 2008 to 2012 and Stat Strip from 2012 to present. The Stat Strip glucose strip technology is based on an amperometric test system with multilayer-gold multielectrode and four-well test strip based on a modified glucose oxidase enzyme method [21]. No calibration codes or lot numbers need to be entered before measurement. The sample volume is 1,2 μL, and the analysis time is 6 s. Stat Strip measures hematocrit and corrects glucose values for abnormal hematocrits. The Stat Strip received clearance from Food and Drug Administration for use in neonatal testing and is intended for in vitro diagnostic use with capillary, venous, and arterial whole blood. It is approved for all hospital areas, including but not limited to critical care, the operating room, inpatient sites, and outpatient sites, such us diabetic clinics [33].

Glucocard X uses a glucose dehydrogenase method. No calibration codes or lot numbers need to be entered before measurement. The sample volume is 0,3 mL, and the analysis time is 5 s [22]. The Glucocard X can be used only with fresh capillary blood samples.

HemoCue uses a modified glucose dehydrogenase method. No calibration codes or lot numbers need to be entered before measurement. The sample volume is 5 μL, and the analysis time is 20-240 s, depending on glucose concentrations.
All the above described meters are calibrated to report results in plasma equivalent [35].

The Cobas 6000 (Roche, Switzerland) is a fully automated clinical chemistry and immunology analyzer for carrying out biochemistry tests from blood serum and plasma. Plasma hexokinase method was used as the laboratory reference method for measuring glucose concentration. Hexokinase catalyzes the phoshporylation of glucose to glucose -6-phosphate. NADPH formation during oxidation to gluconate-6-phosphate reaction is measured photometrically [12] .

The ABL 825 (Radiometer, Denmark) is a fully automated analyzer for potentiometric measurement of pH, pCO_2 , Na^+, K^+, Ca^{++}, Cl^- and amperometric measurement of pO_2, glucose and lactate . The tip of glucose electrode consist the three layer membrane and middle of them is enzyme glucose oxidase membrane. The H_2O_2 formated by the enzyme reaction produces an electrical current. [20].

3.1.2 Samples

From ICU patients two arterial blood samples from each patient were collected according to the routine order for laboratory tests – one sample for biochemistry tests and one sample for blood gas measurement. Biochemistry samples were collected in sodium heparin BD Vacutainer for plasma glucose and other biochemistry tests. Blood gas samples were collected into heparinized Safe Pico (Radiometer, Denmark) syringe for blood gas testing and glucose. No additional blood drawing or finger stick samples were used.
Samples were collected from umbilical arterial catheters into sodium heparin BD Vacutainer and immediately cooled to +4C° to reduce glycolysis. Samples were centrifuged at +4C° according to the manufacturer's recommendation. Blood gas syringes were analyzed immediately after receiving in the laboratory. After blood gas measurements the glucose meter, loaded with a strip, was touched to a drop of top of syringe, as if it were on a patient`s fingertip, and specimen was drawn into the test strip by capillary action to measure the glucose concentration.
One capillary blood sample was collected from NCU patients according to routine order for blood glucose. From one drop of blood glucose concentration was measured by Stat Strip and

HemoCue. If the glucose concentration was below the cut-off value (2,6 mmol/L) an additional capillary sample was collected for comparison with laboratory measurements (ABL 825 blood gas analyzer).
Ten blood samples were selected, after blood gas measurements in the laboratory with different glucose concentrations. The glucose concentration was then measured in each ABL 825 blood gas analyzer, Stat Strip and HemoCue glucose meters.

3.1.3 Quality control

The performance of Cobas 6000 and ABL 825 analyzers were monitored according to the manufacturer`s specifications as part of laboratory requirements for reporting patients results. Control samplesfor different glucose concentration ranges were measured at least once every 24 hours. Stat Strip meters were operated within controls daily in triplicates during five days according to the guidelines [34].

3.1.4 ISO 15197 guideline

The ISO 15197 guideline states that glucose meter measurements should be within ±0,83 mmol/L of the reference result for glucose concentration <4,2 mmol/L and within ±20 % for concentrations ≥ 4,2 mmol/L [24].

3.1.5 Method comparisons

Comparison measurements of the methods were performed by analyzing 41 ICU whole blood samples with the Stat Strip glucose meter and with the ABL 825 blood gas analyzer. The reference method measurement was performed on 20 ICU plasma specimens.
Seventy four NCU whole blood samples on the Stat Strip glucose meter results were compared to those obtained with the the HemoCue glucose meter. Samples with glucose concentrations below 2,6 mmol/L (cut-off value) were additionally analyzed by the laboratory blood gas analyzer for confirmation. Confirmation of test results that are critical for patient health by other method and/or analyzer, is an obligatory procedure for all users, who perform laboratory analyses. After confirmation the physician will decided for immediately treatment of patient with critical test result.
The data of samples for each meter and the Cobas and ABL systems were analyzed to determine the slope, intercept, and correlation coefficient for the data sets.

3.2 Comparison of fertility hormones and tumor markers

3.2.1 Materials and methods

The study was performed over a two months period. We selected altogether over 500 sample results with routine order of hormone tests. After measurements of hormone concentrations with one analyzer the samples were measured again on another. No additional blood drawings were used.

3.2.2 Instrumentation

Cobas 6000 (Roche, Switzerland) analyzer series is a completely selective, modular analytical system of the 2nd generation that has been conceived for the consolidated processing of parameters in clinical chemistry and heterogeneous immunology [35].
Cobas 6000 uses specific biotinylated antibodies. A monoclonal specific antibody labeled with ruthenium complex and streptavidin-coated microparticles react to form a sandwich-structured complex, which is bound to the solid phase. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured on the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier [35].
Immulite 2000 (Siemens, Germany) . is an automated immunoassay analyzer that performs chemiluminescent immunoassays. Immulite 2000 uses specific antibody-coated polystyrene beads as the solid phase. A bead is dispensed into a specially designed reaction tube, which serves as the vessel for the incubation, wash, and signal development processes. After the sample is incubated with an alkaline phosphatase reagent, the reaction mixture is separated from the bead by spinning the reaction tube at high speed along its vertical axis. Four discrete washes occur within seconds, the bead remains in the reaction tube with no residual unbound label. The bound label is then quantified using the dioxetane substrate to produce light. Light is emitted when the chemiluminescent substrate reacts with the alkaline phosphatase bound to the bead. The amount of light emitted is proportional to the analyte originally present in the sample, this light emission is detected by the photomultiplier tube and results are calculated for each sample [36].

3.2.3 Data of samples, method performance data and quality of assays

Table 7 summarizes the number of samples of the method comparison.

Table7*. Number of samples*

Test name	Number of samples
Alpha-fetoprotein	194
Testosterone	138
Luteinizing hormone	104
Follicle stimulating hormone	121
Estradiol	97
Prostate-specific antigen	153
Free prostate specific antigen	114

Data on linearity of each test performed with both analyzers are shown in Table 8.

Table 8*. Linearity intervals of methods*

Quantity	Immulite 2000	Cobas 6000
Alfa-fetoprotein, IU/mL	0,2-300	0,5-1000
Estradiol, pmol/L	73,4-7342	18,4-15781
Luteinizing hormone, U/L	0,10-200	0,10-10000
Testosterone, nmol/L	0,69-55,5	0,087-52,0
Follicle stimulating hormone, U/L	0,1-170	0,1-200
Prostate specific antigen, µg/L	0,003-20	0,003-100
Free prostate specific antigen, µg/L	0,07-25,00	0,010-50,00

In 6 of 7 tests the Cobas analyzer had better linearity intervals than Immulite.

Imprecision data of the assays as estimated from measurements of control samples (commercial samples, recommended for daily internal quality control procedure and produced by the test kit manufacturer) are summarized in Table 9, including the number of control levels by the analyzer`s software.

Table 9. *Quality control data*

Analyzer / Quantity	Immulite 2000		Cobas 6000	
	Mean	CV, %	Mean	CV, %
Alfa-fetoprotein	14,0 IU/mL	6,26	10,2 IU/mL	4,14
	63,1 IU/mL	7,73	51,5 IU/mL	3,99
	134 IU/mL	7,82		
Estradiol	267 pmol/L	11,7	378 pmol/L	4,41
	583 pmol/L	7,45	1894 pmol/L	4,97
	4060 pmol/L	5,73		
Luteinizing hormone	9,06 IU/mL	6,47	12,56 IU/mL	4,13
	19,8 IU/mL	6,17	55,78 IU/mL	4,41
	49,2 IU/mL	8,18		
Testosterone	3,71 nmol/L	7,37	7,27 nmol/L	10,97
	9,85 nmol/L	6,70	20,60 nmol/L	4,38
	27,0 nmol/L	5,46		
Follicle stimulating hormone	8,01 U/L	6,21	16,50 U/L	4,32
	17,0 U/L	4,74	49,30 U/L	5,32
	42,4 U/L	3,99		
Prostate specific antigen	6,93 µg/L	5,07	3,47 µg/L	3,61
	14,3 µg/L	4,23	35,90 µg/L	3,40
Free prostate specific antigen	8,88 µg/L	4,50	0,89 µg/L	3,07
	17,5 µg/L	6,37	8,98 µg/L	2,90

In the comparison of imprecision of immunological tests the Cobas analyzer demonstrated smaller CV% than the Immulite analyzer.

4. Data analysis

Comparison of glucose, hormones and tumor markers were performed by use of the ACB spreadsheet program (version 3.89) [37]. The ACB spreadsheet program is primarily designed for estimating the bias by comparing the results of measuring the analyte concentration in patient samples by two methods.

A scatter plot (see Figure 3 as an example) and relative and absolute difference graphs with optional display of mountain plots are provided. Ordinary linear and Deming regression parameters are calculated. The data set can be partitioned into three parts, which can be useful if different regression modes describe the data at low, medium and high concentrations. If such partitioning is used then each partition can be separately reported. Regression functions and parameters can be calculated separately for each partition and can optionally be displayed. The statistical significance of the comparisons can be evaluated by Student's t-test and Wilcoxon's sign rank test but the final decision on feasibility rests on the mean and relative difference between the results, i.e. a clinical evaluation. Therefore the mean and relative differences between the measurement results need to be considered. The false rejection rate (α) was set to 5 %, corresponding to a confidence level of 95 %. An outlier test (Grubbs' test) was performed on the differences between the results at 5 % level. The Grubbs' test is only performed if the dataset contains less than 100 observations. The slope and intercept of the ordinary linear regression (OLR) and their uncertainties are displayed. If the variances of the measurements by the test and comparative methods are estimated (duplicate results), or entered, the Deming regression with the uncertainty of the slope and intercept will also be calculated.

Imprecision for the laboratory and POCT analysis was evaluated by the analyzer`s software and Microsoft Excel 2002, respectively.

5. Results

5.1 Results of performance of glucose meters

5.1.1 Quality control

Table 10 presents the data on imprecision of the control samples (mean and CV%) characterized by the 95 % confidence interval and the respective ISO 15197 requirements and evaluation of compliance with ISO 15197 criteria.

Table10. *Quality control data and comparison with ISO 15197*

Mean glucose value, mmol/L	CV %	95 % CI, mmol/L	ISO 15197	Compliant with ISO 15197 criteria?
3,3	6,1	3,4±0,42 mmol/L	3,4 ±0,83 mmol/L	yes
6,4	3,4	6,0±0,42 mmol/L	6,0 ±1,20 mmol/L	yes
16,1	5,4	15,7±1,68 mmol/L	15,7 ±3,14 mmol/L	yes

5.1.2 Method correlations

The linear regression analysis demonstrated a slope of 1,18 and the Stat Strip glucose meter had the lowest mean bias (-0,160 mmol/L) compared with the laboratory hexokinase method. The mean difference of the Stat Strip glucose meter results compared with those of the ABL 825 was -0,037 mmol/L and a slope 1,01. The results demonstrate that Stat Strip glucose meter and the laboratory methods had no significant bias: p = 0,545 for the hexokinase method and p = 0,723 for the ABL method.

HemoCue glucose meter had significantly lower results compared to Nova Stat Strip glucose meter (p = 0,001). Linear regression analysis demonstrated a slope of 0,41/0,95/0,53 and an intercept of 1,45/-0,75/1,23 mmol/L at the three concentration intervals 1,8-4,3/4,4-5,0/5,1-10,0 mmol/L, respectively. The maximum difference found was 65 % (-2,2 mmol/L) at the value 4,5 mmol/L. The mean difference was 1,0 mmol/L.

Table 11 presents data of the method comparison of glucose: name of glucose meter, slope, intercept and mean difference expressed in unit and %.

Table 11.Correlation data for the POCT glucose meters versus the laboratory analyzer ABL 825

Meter	Slope	Intercept	Mean Difference, mmol/L	Mean Difference, %
HemoCue	1,07	-0,89	-0,422	-7,1
StatStrip	0,95	0,19	-0,156	-2,4

The HemoCue glucose meter demonstrated a significant negative bias (-7,1 %) compared to the laboratory method (p = 0,034). The Stat Strip glucose meter had no significant bias compared to the laboratory method (p = 0,428).

Comparison of the glucose meters at the cut-off value (Table 12) showed that the number of samples where the glucose concentrations less than 2,6 mmol/L were found was 12 with the HemoCue glucose meter while only 2 samples with glucose concentration below 2,6 mmol/L were found with the Stat Strip glucose meter. The 2 low results obtained by the Stat Strip/HemoCue meters were also confirmed by the laboratory analysis. At the same time 10 results from HemoCue glucose meter had glucose value above 2,6 mmol/L but were not confirmed by the laboratory results.

Table 12*.Comparison of glucose at the cut-off value (≤2,6 mmol/L)*

Sample	HemoCue	Stat Strip	ABL 825	HemoCue/StatStrip comparison with ABL?
1	2,3	2,8	2,7	No/Yes
2	2,5	3,3	3,4	No/Yes
3	2,3	3,8	3,6	No/Yes
4	2,2	3,3	3,1	No/Yes
5	2,2	3,2	3,0	No/Yes
6	2,5	3,3	3,1	No/Yes
7	1,4	1,8	2,0	Yes/Yes
8	2,5	3,4	3,7	No/Yes
9	2,2	3,6	3,5	No/Yes
10	2,3	4,4	4,5	No/Yes
11	2,4	3,6	3,2	No/Yes
12	1,9	2,0	2,0	Yes/Yes

5.2Results of comparison of immunological tests

Table 13 presents the data of the method comparison of immunological tests: measurand, unit, number of samples, partitioning of results, slope, intercept and mean difference.

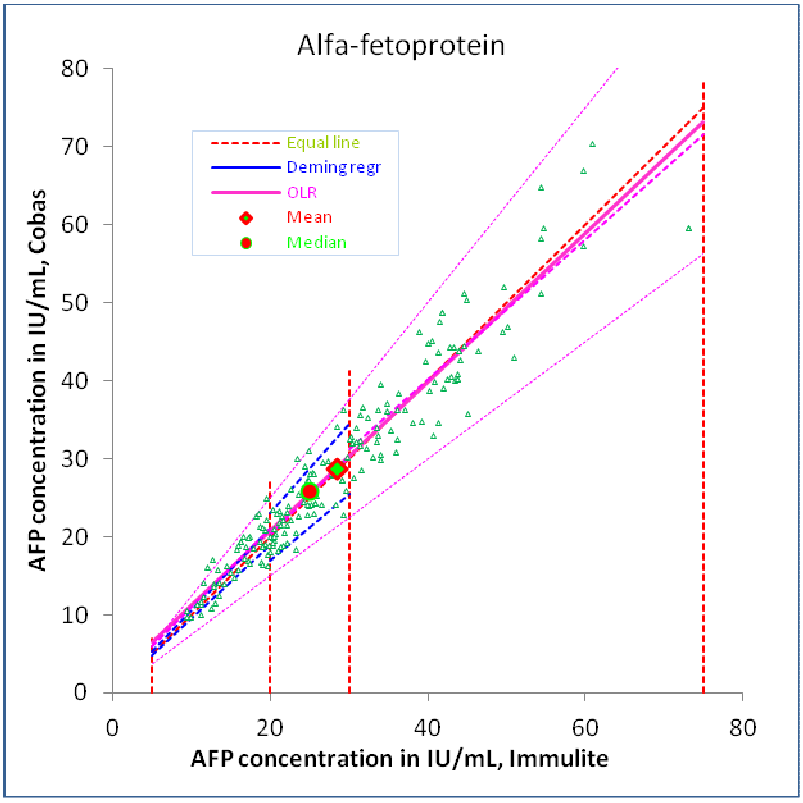
Table 13. *Correlation data of immunological tests for the Immulite analyzer versus the Cobas analyzer.*

Measurand	n	Partitioning of results	Slope	Intercept	Mean difference, %
Alfa-fetoprotein, IU/mL	51	5-20	1,00	1,02	6,1
	66	20-30	1,02	-0,18	0,7
	73	30-75	0,90	4,30	0,0
Estradiol, pmol/L	11	18,4-73,4	NA*	NA*	NA*
	59	73,5-210	0,99	-13,11	-21
	27	210-3000	1,19	3,88	12,8
Luteinizing hormone, LH, U/L	39	0,0-3,0	1,49	0,01	33,2
	40	3,0-9,0	1,16	0,75	27,3
	25	9,0-150,0	1,31	-3,64	5,8
Testosterone, nmol/L	29	0,087-0,69	NA*	NA*	NA*
	33	0,7-5,0	0,95	0,16	3,6
	28	5,0-11,0	1,37	-1,38	16,6
	48	11,0-55,0	1,16	0,47	16,3
Follicle stimulating hormone, U/L	24	0,0-3,5	1,06	-0,14	-0,9
	75	3,5-12,5	0,92	0,06	-8,3
	22	12,5-95,0	0,89	-0,02	-11,6
Prostate specific antigen, µg/L	40	0,003-0,9	0,89	-0,02	-32,9
	68	0,9-4,0	0,96	-0,04	-7,2
	45	4,0-300,0	0,94	0,53	-4,9
Free prostate specific antigen, µg/L	21	0,00-0,070	NA*	NA*	NA*
	49	0,071-0,70	0,90	0,15	34,4
	44	0,70-50,0	1,11	-0,10	0,1

* NA-not available to calculate, Cobas assays has a lower detection limit than Immulite assays, see table 8.

The comparison results of alfa-fetoprotein on Cobas and Immulite platforms, the mean, median and equal line are demonstrated in Figure 3. Alfa-fetoprotein comparison had one of the highest number of samples for all data and for every interval.

Figure 3. *Scatter plot of comparison, alfa-fetoprotein example.*



This study demonstrated that harmonization may be accomplished by establishing calibration traceability to the mean values for a patient samples. An example of recalibration for the luteinizing hormone (LH) is presented in Tables 14 and 15.

Table 14. Recalculation of the previous results (measured in laboratory by the Immulite analyzer before 10.2012) for harmonization with the new Cobas system. P-LH is used as an example and the recalculation formula is from table 13..

Interval of value Siemens	Recalculation formula	Example Siemens	New result Roche	**Enter LH value** Siemens	**Corrected value** Roche
0,0-3,0 U/L	1,49 *x* (LH)+0,01	1,5	2,2	x	
3,0-9,0 U/L	1,16 *x* (LH)+0,75	5	6,6	x	

9,0-150 U/L	1,31(LH)-3,64	50	62	x	

Table 15. Current results (measured in Laboratory since 10.2012 by Roche) and recalculation used for harmonization with the old system (Siemens)

Interval of value Roche	Recalculation formula	Example Roche	New result Siemens	**Enter LH value** Roche	**Corrected value** Siemens
0,0-4,5 U/L	0,67 *x* (LH)	2,2	1,5	x	
4,6-11,2 U/L	0,86 *x* (LH)-0,65	6,6	5,0	x	
11,3-193 U/L	0,76 *x* (LH)+2,8	62	50	x	

6. Summary

It was demonstrated that the Stat Strip glucose meter meets the ISO 15197 performance criteria and gives results that are in close correlation to the laboratory methods. In contrast, the HemoCue glucose meter was inadequate for monitoring glucose concentrations in neonates. Stat Strip glucose meter is a true and precise alternative for near-patient testing in neonatal setting.

Comparison of patient results gives us a possibility to recalculate results from different measurement procedures and thus estimate if separate intervals are needed. The uncertainty attached to the end result will increase but it quantification is not addressed in this report. Determination of the tumor markers is intended for use as an aid to the management of patients with tumor and monitor patients after treatment. Determination of fertility hormones is used for management fertility status of women and men and monitor patients after treatment.

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8. Kokkuvõte

Agnes Ivanov

Rutiinmeetodite tulemuste võrreldavus ja jälgitavus meditsiinilaboris

Käesoleva töö eesmärgiks oli võrrelda Tartu Ülikooli Kliinikumis kasutatavate laboratoorseid meetodeid, hinnata nende meetodite erinevust ja töötada välja süsteem meetodite erinevuse vähendamiseks.
Töö esimeses osas antakse kirjanduslik lühiülevaade laborianalüüside metroloogilisest jälgitavusest ja analüüside jälgitavuse klassifikatsioonist. Tuuakse välja laboratoorsete meetodite erinevuse põhjused.
Glükoosi analüüsi näitel võrreldi haigla laboris kasutusel olevaid meetodeid nende meetoditega, mis on kasutusel haigla osakonnas patsiendi voodi juures (POCT).
Immunoloogiliste suguhormoonide ja kasvajate markerite meetodeid võrreldi varem kasutusel olnud laboris Immulite 2000 analüsaatorit ja 2012 aastal kasutusele võetud Cobas 6000 analüsaatorit.
Töös on toodud võrdluste andmed ja antud hinnang. Välja on töötatud eeskirjad ühtede seadmete tulemuste ümberarvutamiseks teiste seadmete peale analüüside tulemuste harmoniseerimiseks.
Väljatöötatud metoodikaga on võimalik võrrelda varem saadud analüüsi tulemuse, mis oli teostatud Immulite 2000 analüsaatoril käesoleval ajal saadud tulemustega, mis on teostatud Cobas 6000 analüsaatoril. Analüüside tulemuste tegelik erinevus peegeldab muutusi patsiendi bioloogilises seisundis ning annab võimaluse raviarstile teha õige raviotsuse patsiendi ravimisel ja jälgimisel.

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