XIII Baltic Congress in Laboratory Medicine

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XIII Baltic Congress in Laboratory Medicine

Tartu, Estonia
May 12–14, 2016

Abstracts

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Welcome to the XIII Baltic Congress in Laboratory Medicine!

Dear Colleagues,

On behalf of the Baltic Association of Laboratory Medicine (BALM) and the Organizing Committee, I would like to express my great pleasure inviting you to participate in the XIII Baltic Congress in Laboratory Medicine. This important event will be held in Tartu, Estonia, on May 12–14, 2016 at the Dorpat Conference Centre.

The Congress will be held under the auspices of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and with the generous support of the IFCC Visiting Lecturer Programme.

The BALM Congress Tartu 2016 is going to be an invigorative event reflecting the state of art and directions of laboratory medicine at the time of a changing health care environment. The Congress will focus on topics of how laboratory medicine can help clinicians to treat patients in a better way.

The Scientific Committee has worked hard to present a challenging scientific programme in the form of plenary lectures, scientific sessions, workshops and a poster session. Baltic, Nordic and international speakers will bring along their best knowledge for sharing their ideas. Both young and experienced colleagues will present posters on their research findings. Partners from the diagnostic industry will introduce new developments and ideas at a commercial exhibition and in presentations.

The BALM Congress Tartu 2016 will offer an excellent opportunity to meet colleagues, to gather knowledge, and to share information. We hope that it will also help to strengthen cooperation and communication between Baltic and international colleagues.

Tartu, called also the Athens of the River Emajõgi, is a beautiful university town, and a modern city with rich historical heritage and culture. A walk in the Old Town and in the Town Hall Square, as well as the vibrant spring atmosphere of this student town with its sightseeing sites, like the *Ahhaa* Science Centre, the Vanemuine theatre and the university buildings, will undoubtedly be an enjoyable experience.

Welcome to Tartu 2016!

Dr Karel Tomberg
President of the Congress

Committees

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Baltic Congresses in Laboratory Medicine

1st	1992 Tartu, Estonia
2nd	1994 Vilnius, Lithuania
3rd	1996 Jurmala, Latvia
4th	1998 Tartu, Estonia
5th	2000 Vilnius, Lithuania
6th	2002 Riga, Latvia
7th	2004 Pärnu, Estonia
8th	2006 Vilnius, Lithuania
9th	2008 Jurmala, Latvia
10th	2010 Tallinn, Estonia
11th	2012 Vilnius, Lithuania
12th	2014 Riga, Latvia
13th	2016 Tartu, Estonia

Congress Programme

THURSDAY, MAY 12		
18.00–19.30	**Opening**	**Hall STRUVE**
	Greetings	Karel Tomberg, Estonia
18.15–18.45	Developments in Estonian medicine in last 25 years	Urmas Siigur, Estonia
18.45–19.15	Cooperation possibilities with IFCC. Update on the IFCC e-academy	Janet Smith, UK
19.30–23.00	**Welcome Party**	**Science Center AHHAA**

FRIDAY, MAY 13		
8.30–9.00	**Coffee break, posters**	
9.00–9.45	**Plenary session**	**Hall STRUVE**
	How to set performance specifications in laboratory medicine	Sverre Sandberg, Norway
9.50–11.30	**Morning sessions**	
Session 1	**Laboratory management**	**Hall STRUVE**
9.50–10.20	Improving the efficiency of clinical laboratory services	Janet Smith, UK
10.20–10.50	Laboratory management through consolidation / automation	Herbert Stekel, Austria
10.50–11.10	Laboratory consolidation: a Finnish example of networking	Ari Miettinen, Finland
11.10–11.30	Quality indicators in laboratory medicine: from theory to practice	Agnes Ivanov, Estonia
Session 2	**Laboratory haematology**	**Hall BAER**
9.50–10.20	Monitoring new oral anticoagulants	Lotta Joutsi-Korhonen, Finland
10.20–10.40	Permanent fight between Owren and Quick	Valdas Banys, Lithuania
10.40–11.00	Screening for hemoglobinopathies	Marika Pikta, Estonia
11.00–11.20	Relationship between clinicians and specialists of laboratory medicine in diagnostics of haemostasis disorders	Daiva Urbonienė, Lithuania
9.50–11.30	**Workshop on the microscopy diagnostics of urogenital tract diseases**	**Hall PETERSON**
11.30–12.30	**Coffee break, posters, commercial presentations**	
12.00–12.30	**Commercial presentation 1, radiometer medical aps**	**Hall STRUVE**
	Acute care – preanalytical and analytical aspects of blood gas and biomarker testing	Irakli Jaliashvili, Denmark
12.00–12.30	**Commercial presentation 2, Roche Diagnostics**	**Hall BAER**
	Laboratory and Quality Value in Heart Failure Management	Gábor L. Kovács, Hungary
12.30–14.00	**Mid-day sessions**	
Session 3	**Quality assurance**	**Hall STRUVE**
12.30–13.00	Quality system for GPs; Norwegian example	Sverre Sandberg, Norway
13.00–13.30	How is EFLM WG-PRE contributing to the improvement of preanalytical phase quality in Europe	Ana-Maria Šimundic, Croatia
13.30–14.00	Critical risk results in Europe	Eva Ajzner, Hungary

PROGRAMME

Session 4	Biomarkers	Hall BAER
12.30–12.50	New biomarkers for pregnancy	Maris Laan, Estonia
12.50–13.20	New trends in cardiovascular laboratory	Kari Pulkki, Finland
13.20–13.40	Biomarkers in chronic kidney disease	Erika Skrodenienė, Lithuania
13.40–14.00	New biomarkers of articular cartilage in patients with osteoarthritis	Agu Tamm, Estonia
12.30–14.00	**Workshop on the microscopy diagnostics of urogenital tract diseases (continued)**	**Hall PETERSON**
14:00–15.30	**Lunch, posters, commercial presentations**	
14.30–15.00	**Commercial presentation 3, Nova Biomedical**	**Hall STRUVE**
	New era in point-of-care glucose testing in the hospital: regulatory changes and clinical significance	Germano Ferrari, UK
14.15–15.00	**Commercial presentation 4, Abbott Laboratories**	**Hall BAER**
	Diagnostic and prognostic value of high sensitive troponin I	Claudio Galli, Italy
15.00–15.30	**Commercial presentation 5, Thermo Fisher Scientific (Phadia)**	**Hall STRUVE**
	The use of allergen component IgE testing	Peter Csonka, Finland
15.00–15.30	**Commercial presentation 6, Triolab**	**Hall BAER**
	The next generation in rapid, point of care testing	Johanna Galinski, Germany

15.30–17.00	**Afternoon sessions**	
Session 5	**New trends in laboratory medicine. Best abstracts**	**Hall STRUVE**
15.30–16.00	Patient-focused laboratory medicine. The Lab4Patients Horizon 2020 Project	Wytze Oosterhuis, Netherlands
16.00–16.15	Syndrome based test panels in hospital laboratory	Karel Tomberg, Estonia
16.15–16.30	Comparative whole genome hybridisation methods in molecular diagnostics	Beata Aleksiūnienė, Lithuania
	Best abstract presentations	
16.30–16.40	Diagnostic of tick-borne encephalitis virus infection in acute antibody negative samples by real-time PCR	Irina Golovljova, Estonia
16.40–16.50	Red cell indices and C-reactive protein in pediatric patients are closely related	Sergey Nikulshin, Latvia
16.50–17.00	Actionable mutations in the whole-genome sequenced gene donors of Estonian Biobank	Neeme Tõnisson, Estonia

Session 6	Sepsis management	Hall BAER
15.30–15.50	Sepsis management audit in Estonia	Pille Märtin, Estonia
15.50–16.10	Mass-spectrometry for bloodstream pathogen identification in routine practice	Marina Ivanova, Estonia
16.10–16.25	First data on invasive bacteria antimicrobial resistance from mandatory AMR surveillance program in Lithuania	Jolanta Miciuleviciene, Lithuania
16.25–16.40	Diagnostic possibilities of fungal infections	Helle Järv, Estonia
15.30–17.00	**Workshop on method verification**	**Hall PETERSON**
19.30–23.00	**Congress banquet**	**Restaurant ATLANTIS**

SATURDAY, MAY 14		
8.00–8.30	**Coffee break, posters**	
8.30–9.00	**Plenary session**	**Hall STRUVE**
	Laboratory medicine in Europe	Wytze Oosterhuis, Netherlands

9.00–11.00	**Morning sessions**	
Session 7	**LIS and electronic health**	**Hall STRUVE**
9.00–9.40	LIS – the basics	Herbert Stekel, Austria
9.40–10.00	LIS and eHealth in Finland	Hannu Honkala, Finland
10.00–10.20	eHealth in Estonia	Viljar Pallo, Estonia
10.20–10.40	eHealth in Lithuania	Andrius Šimėnas, Lithuania
10.40–10.55	Standardization of lab tests in Lithuania	Dalius Vitkus, Lithuania
Session 8	**Sexually transmitted diseases**	**Hall BAER**
9.00–9.30	N gonorrhoeae antibiotic resistance and new diagnostic guidelines	Magnus Unemo, Sweden
9.30–10.00	HPV based screening, is it worth for fight?	Mari Nygard, Norway
10.00–10.30	Interpretation of syphilis serodiagnostic tests: looking from the clinician`s side	Peter Karl Kohl, Germany
10.30–10.40	Same algorithms for the labs and clinicians? Estonian experience	Raili Randoja, Estonia
10.40–10.50	Application of syphilis laboratory diagnostics in Latvia	Gatis Pakarna, Latvia
10.50–11.00	Sexually transmitted infections in Lithuania	Vesta Kučinskienė, Lithuania
9.00–11.00	**Workshop on porphyria diagnostics**	**Hall PETERSON**
11.00–12.00	Coffee break, posters, commercial presentations	
11.30–12.00	**Commercial presentation 7, Siemens Healthcare**	**Hall STRUVE**
	How to improve the clinical diagnosis in emergency departments in hospitals	Per Simonsson, Sweden
11.30–12.00	**Commercial presentation 8, Thermo Fisher Scientific (BRAHMS)**	**Hall BAER**
	Procalcitonin and the antibiotic management	János Fazakas, Hungary
12.00–13.45	**Mid-day sessions**	
Session 9	**Cancer: diagnostic markers, cure**	**Hall STRUVE**
12.00–13.00	Diagnostic markers for cancer	Petra Stieber, Germany
13.00–13.15	Colorectal cancer screening: new developments in Europe	Marcis Leja, Latvia
13.15–13.30	Colorectal cancer, new immunotherapy	Jüri Laasik, Estonia
13.30–13.45	Lymphocyte subsets and serological markers in melanoma patients treated with ECHO7 virus	Simona Donina, Latvia
Session 10	**Antibiotic resistance management**	**Hall BAER**
12.00–12.30	Emergence and spread of antibiotic resistance – not only a medical problem	Tanel Tenson, Estonia
12.30–12.45	Epidemiology of AB resistant gram-negative bacteria in the Baltic Sea region	Anastasia Pavelkovich, Estonia
12.45–13.00	Management of antibiotic resistance at the largest university hospital of Lithuania	Asta Dambrauskienė, Lithuania
13.00–13.15	PCR-based identification and serotyping of streptococcus pneumoniae strains and antibacterial sensitivity assessment in Latvia	Oksana Savicka, Latvia
13.15–13.30	Detection of antimicrobial resistance of Salmonella and Campylobacter according to the EU protocol	Solvita Selderiņa, Latvia
13.45–13.50	**BALM 2016 Best Poster Award**	**Hall STRUVE**
13.50–14.00	**Closing Ceremony**	**Hall STRUVE**

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PL-1. Cooperation possibilities with IFCC. Update on the IFCC e-academy

Janet Smith[1]

The mission statement of IFCC is to be the leading organisation in the field of Laboratory Medicine worldwide. Its membership reflects this aim, 90 full member national learned societies and six affiliate member societies that are most strongly affiliated to six Regional federations as well as 46 corporate institutions.

The organisation of IFCC is effected through its Council, comprising representatives of all National Societies and its Executive, membership of which is elected triennially. Council and Executive oversee three operational Divisions, the Scientific Division (SD), the Education and Management Division (EMD) and the Communications and Publications Divisions (CPD), specialist committees and special task forces. Each Division has its own committees and working groups. Members of the committees and working groups are nominated by National Societies and Corporate Members; this provides many opportunities for individuals to contribute to the work of IFCC. National Societies; also individuals can take advantage of IFCC special projects such as the Visiting Lecturer Programme (VLP) the Professional Exchange Programme (PEP) the Speakers' Bureau and the Register of Experts.

In recent years distance learning has become a key initiative of IFCC; the EMD Committee on Distance Learning (C-DL) and the CPD Committee on the Internet and e-Learning (C-IeL) have been collaborating to develop the IFCC eAcademy, which will produce high quality educational modules available to its membership. The eAcademy will provide a resource for individuals in their training and CPD requirements as well as for those in the planning and organisation of educational programmes.

The eAcademy is a Learning Management System which uses a curriculum based approach to catalogue and access educational materials; it contains linked presentations, webinars and other educational material managed through the Umbraco content management system. There are three phases in its development, the first was launched in Paris in 2015. Preliminary work on content has concentrated on some of the topics highlighted as priority needs by National Societies.

Two approaches are being used to acquire high quality material for the eAcademy. The first is using the present.me software, which enables powerpoint slides to be coupled with video and voiceover, to prepare short modules covering specific topics. The second is to identify interesting presentations at IFCC and National Society scientific meetings for recording and inclusion in the eAcademy. Appropriate presentations are identified from the published programmes for upcoming events. We need the support and help of National Societies to cooperate with us in arranging the recordings. It is through the generous financial support of Siemens that we are able to finance this approach.

A further part of the project is to identify high quality material published by other organisations and we are working with these materials, including EFLM and AACB, to allow link to distance learning modules on their websites.

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PL-2. Proposal for the modification of the conventional model for establishing performance specifications

Wytze P. Oosterhuis[1], Sverre Sandberg[2]

Abstract: Appropriate quality of test results is fundamental to the work of the medical laboratory. How to define the level of quality needed is a question that has been subject to much debate. Quality specifications have been defined based on criteria derived from the clinical applicability, validity of reference limits and reference change values, state-of-the-art performance, and other criteria, depending on the clinical application or technical characteristics of the measurement. Quality specifications are often expressed as the total error allowable (TE_A) – the total amount of error that is medically, administratively, or legally acceptable. Following the TE_A concept, bias and imprecision are combined into one number representing the "maximum allowable" error in the result. The commonly accepted method for calculation of the allowable error based on biological variation might, however, have room for improvement. In the present paper, we discuss common theories on the determination of quality specifications. A model is presented that combines the stateof-the-art with biological variation for the calculation of performance specifications. The validity of reference limits and reference change values are central to this model. The model applies to almost any test if biological variation can be defined. A pragmatic method for the design of internal quality control is presented.

INTRODUCTION

A good quality of test results is fundamental to the work of the medical laboratory. How to define the level of quality needed is a question that has been subject to much debate, and more than one consensus agreement has been reached to define quality specifications [1–4]. Quality specifications have been defined on the basis of criteria derived from the clinical applicability, validity of reference limits and reference change values, state-of-the-art performance, and other criteria, depending on the application and the characteristics of the test.

Quality specifications are often expressed as the total error allowable (TE_A) – the total amount of error that is medically, administratively, or legally acceptable. Following the TE_A concept, bias and imprecision are combined into one number representing the "allowable" error in the result. Internal quality control (IQC) procedures, as well as external quality assessment (EQA) can be shaped according to the TE_A of the analyte [5]. The Six Sigma concept is also linked to TE_A, as the sigma value is derived from this entity [5, 6]. "Sigma-metrics" are valuable to "normalize" quality to a common scale.

The commonly accepted method for calculation of the allowable error based on biological variation might, however, have room for improvement. The addition of the bias and imprecision terms according to this method has been shown to overestimate TE_A [7]. In the present paper, we discuss the common theories on the determination of quality specifications. A modified model for the calculation of quality specifications is presented. The validity of reference limits and reference change values are central to this model that is a modification of existing models (further called the "modified model") [8–10].

Cited in: Sandberg S, Fraser CG, Horvath AR, et al. Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem Lab Med 2015;53:833–5.

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Keywords:
allowable error, analytical variation, biological variation, quality specifications, total error

ANALYTICAL QUALITY SPECIFICATIONS

According to the new consensus [1], quality specifications for clinical applicability should preferably be based on clinical outcome, which for most situations is the same as specifications based on decision levels and evaluation of tolerable false-positive (and false-negative) results. It was, however, acknowledged that, in many cases, this is difficult or even impossible to

achieve as for most analytes decision levels cannot be unambiguously defined. According to the consensus document, the preferred alternative approach for these measurands is to derive specifications from biological variation. It should be noted that there is a fundamental difference between these methods, as the specifications based on biological variation are not related to clinical needs but tries to minimize the "signal-to-noise" ratio between analytical variation and the (natural) biological variation.

Analytical performance specifications based on biological variation are now broadly used in clinical chemistry, whereas in clinical guidelines, performance based on clinical criteria is, in most cases, preferred [11, 12].

Cotlove et al. [13] proposed that the tolerable analytical variation (analytical standard deviation) should be less than half of the total biological variation:

$$CV_A < 0.5CV_B \text{ } (CV_B = \text{coefficient of variation, total biological}). \quad (1)$$

Harris [14] then proposed that quality specifications for individual monitoring should be calculated using the formula

$$CV_A < 0.5CV_I \text{ } (CV_I = \text{coefficient of variation, within subject}). \quad (2)$$

For medical diagnosis, the total biological variation was used. In the case of monitoring to detect trends in the results from an individual over a period of time, the within-subject CV_I is used instead of the total biological variation. This strategy was adopted by the College of American Pathologists at the 1976 Aspen Conference [3] and by the Subcommittee on Analytical Goals in Clinical Chemistry of the World Association of Societies of Pathology in London in 1978 [4, 8].

DEFINITION OF BIAS AND IMPRECISION

Mathematically, analytical bias is clearly different from imprecision. Bias or systematic measurement error is defined as an error that in replicate measurements remains constant or varies in a predictable manner [15]. However, it is also stated that, "Systematic measurement error, and its causes, can be known or unknown. A correction can be applied to compensate for a known systematic measurement error." In practice, the distinction between bias and imprecision is, however, less clear. "Systematic" implies a certain time period. As Klee [16] pointed out, bias tends to be dependent on the time interval considered. In this paper, bias is used for the net shift in test values, relative to the set point of the assay when the reference data on patients were collected. Although bias should be removed when possible, in some circumstances, bias is inevitably encountered, such as systematic differences between analyzers measuring the same analyte.

TOTAL ERROR CONCEPT

The total error (TE) is an expression of the total deviation of the test result from the true value. Westgard et al. [17] presented this TE concept using the argument that physicians think rather in terms of the total analytical error, which includes both random and systematic components.

The TE limits are defined by a maximum percentage of test results, generally taken as 5%, that exceeds this limit (one-sided). For example, assume the true value of a plasma glucose measurement is 9 mmol/L and assume the TE, calculated from actual bias and imprecision, is 10%. In that case, there is up to a 5% chance that this actual result will exceed the TE limit. This means that the probability that the true result will be < 8.1 mmol/L or will be > 9.9 mmol/L will each be 5%. Whether this result meets the quality criteria depends on the specification of the quality limits (see below).

The basic expression most generally used for calculation of the TE is [17]

$$TE = \text{bias} + Z \times CV_A. \quad (3)$$

The Z-value is generally taken as 1.65 (95% one-sided) (Note 1).

TOTAL ERROR ALLOWABLE (TE_A)

As shown above, TE can be calculated from actual bias and imprecision when these are known. However, when a limit for TE is predefined (the total error allowable, TE_A), the maximum allowable bias and imprecision can be derived for the acceptable analytical performance. Medical decision levels should be specified, at which concentration the performance of a method is critical. Just as an example, one decision level for glucose could be at 2.8 mmol/L with a TE_A of 20% (0.56 mmol/L). The maximum allowable bias and imprecision can then be calculated using

$$TE_A = \text{bias} + Z \times CV_A. \quad (4)$$

Many combinations of bias and imprecision can meet the limit set by the value selected for TE_A. In our example, the extreme values are bias = 20% (with CV_A = 0) and CV_A = (20/1.65) = 12.1%. Bias and imprecision have a linear (inverse) relationship: a higher bias requires a low imprecision, a high imprecision a low bias. As will be shown below, this model is valid only when biological variation does not play a role.

QUALITY SPECIFICATIONS BASED ON BIOLOGICAL VARIATION

Quality specifications should preferably be based on clinical outcome. The specification of TE_A is not always as straightforward as mentioned before. An alternative is to derive these specifications from biological variation [1].

How can this be achieved? Allowable imprecision and bias had been defined as follows:

– Allowable imprecision $CV_A < 0.5CV_I$ [14].
– Allowable bias $< 0.25(CV_I^2 + CV_G^2)^{1/2}$ [8].

In the case of EQA, these specifications should be fulfilled separately and EQA schemes could be designed accordingly. Fraser and Hyltoft Petersen [18] proposed that in case only a single determination of each survey material is used or allowed, the 95% acceptance range for each laboratory from the target value was proposed to be the sum of both values:

$$95\% \text{ acceptance range} = \text{target value} \pm [1.65(0.5CV_I) + 0.25(CV_I^2 + CV_G^2)^{1/2}].$$

In terms of the TE_A:

$$TE_A = 1.65(0.5CV_I) + 0.25(CV_I^2 + CV_G^2)^{1/2}. \quad (5)$$

TWO FLAWS IN THE CONVENTIONAL MODEL

Although the purpose of expression (5) was the application in EQA, it is commonly accepted and used for other purposes outside EQA, such as the identification of appropriate limits for IQC [5, 19].

As shown above, the quality specification for imprecision is, in general, $CV_A < 0.5CV_B$. In the case of diagnosis, this can be written as $CV_A < 0.5(CV_I^2 + CV_G^2)^{1/2}$. In case of monitoring, only the within-subject variation is included: $CV_A < 0.5CV_I$.

The maximum allowable bias was derived as $0.25CV_B$ or $0.25(CV_I^2 + CV_G^2)^{1/2}$ [8]. It should be noticed, however, that in the conventional model, this bias term is applied in the case of *monitoring* although this expression had been derived from a reference value model and only applies to *diagnosis*. For that reason, in the case of monitoring, we applied in the present study the reference change value model that is only based on CV_I and not on CV_G [9, 10, 20].

Secondly, it has been a pragmatic solution proposed for the use in EQA to add both maxima of allowable bias and imprecision to obtain TE_A as in Eq. (5). The theoretical basis for this is, however, lacking, as two "maximum" errors are added, each allowing 5% of the test results exceeding the limit, and only valid under the mutual exclusive assumptions of zero bias and zero imprecision, respectively. The sum will allow an increase of the percentage of test results exceeding the predefined limits [7].

What could be a rational and correct alternative to combine the effects of bias and imprecision on patient test results?

THEORETICAL MODELS FOR QUALITY SPECIFICATIONS

Several models have been developed to derive maximal bias and imprecision based on reference values and the maximum number of false positives [8, 16]. The model presented here is the model according to Gowans et al. [8].

MODEL OF GOWANS (APPENDIX 1)

The model of Gowans et al. [8] (here referred to as the model of Gowans) is based on the influence of bias and imprecision on the proportion of results outside reference limits. Performance specifications were derived from the maximum number of results outside the reference limits. In the model of Gowans, bias and imprecision are combined into one model. The influence on the false-positive rate is calculated based on a Gaussian distribution.

Owing to the effects of bias, imprecision, or a combination of both, more cases will be outside the reference limits. Instead of the usual 2.5% outside a reference limit at 1.96 SD, a maximum of 4.6% (based on the IFCC guideline on reference values [21]) outside the same limits was assumed to be acceptable. Thus, Gowans' model allows a maximum increase of 84% (Note 2) in false-positive results.

Following Gowans' model, a curve can be calculated that defines the maximum bias and imprecision with 4.6% of results outside the reference limit. The maximum bias and imprecision at the extreme ends of this curve are (see Appendix 1) discussed below.

The maximum allowable error was calculated as follows:

– Maximum bias (when imprecision = 0) = $0.275CV_B$.
– Maximum imprecision (when bias = 0) = $0.597CV_B$.

where CV_B is the total biological variation (in this model, not further specified with respect to CV_I and CV_G).

Between these two extremes, a curve describes the combination of bias and imprecision such that the condition is fulfilled: 4.6% of the results outside the reference limits (one-sided) (Figure 1) (Note 3).

According to Gowans' model, the maximum allowable bias ($0.275CV_B$) only applies when CV_A is minimal (the hypothetical situation with $CV_A = 0$). On the other

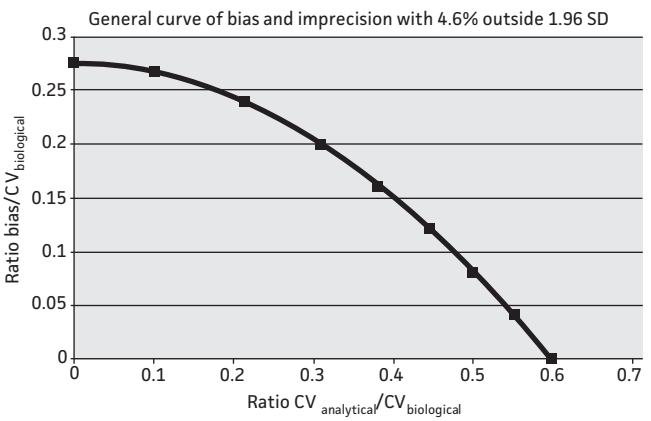

Figure 1: Model according to Gowans et al. [8].
Curved relation between bias and imprecision that describes the combinations of bias and imprecision with 4.6% of the results outside the upper reference limit.

hand, when bias is minimal (bias = 0), the allowed CV_A is at a maximum ($CV_A = 0.597CV_B$). This concept is clearly not in accordance with the model proposed by Fraser and Hyltoft Petersen, mentioned above, where maximum bias and maximum imprecision are summed in the expression of TE_A.

It is important to note that TE_A in Gowans' model is not a constant but varies from $0.275CV_B$ (at $CV_A = 0$) to 1.65 ($0.597CV_B$) (at bias = 0, with Z-factor = 1.65). See also Appendix 1: Gowans for calculation of maximum bias and imprecision of creatine kinase (CK) and sodium, and Table 1.

WHY WAS ANALYTICAL VARIATION NOT INCLUDED IN THE DEFINITION OF PERFORMANCE SPECIFICATIONS?

The starting point of the models of Gowans and others like the model of Klee [16] is the validity of reference limits. The analytical performance specifications of the tests are derived from this concept. Gowans' model has the same assumption as the model of Klee: both define the reference limits without taking the analytical variation into account. This is clearly not the situation in common practice, as reference limits include analytical variation.

Why was this definition of reference limits used without inclusion of the analytical variation? The reason can be understood from the paper itself [8]: by including the analytical variation in the reference interval, the performance specification for analytical variation will, in part, be determined by the analytical variation itself.

DIFFERENT APPLICATIONS OF QUALITY STANDARDS

How to solve the problem of defining performance specifications without this circular argument involved in defining, applying, and controlling analytical quality?

Three different quality objectives should be separated at this point. The *first* is the achievement of the minimum analytical performance needed for clinical use of a test – answering the question: is the quality of the assay acceptable for routine use? This is a concept of the clinical utility of a test. For this decision, different criteria can be applied here, as covered by consensus-based quality specifications, e.g., $CV_A < 0.5CV_I$.

The *second* quality objective is the achievement of the minimum analytical performance to maintain the validity of the reference limits (or, in the case of monitoring, reference change values), corresponding to the situation at the time the test was taken into use.

Third is the inclusion of IQC into the concept. To maintain the minimum analytical performance, some extra quality margin is needed due to the limited sensitivity for bias and imprecision of IQC procedures (see below).

In the case of sodium, the analytical variation is generally higher than the biological variation. The reference limits are, for the greater part, determined by the analytical variation, not by the biological variation. With quality criteria based on the presented models, the test would fail these quality requirements (in theory, this could be overcome by replicate measurements). The quality could also be related to the state-of-the-art performance; however, this would mean that the required quality is not based anymore on any theoretical model, and the validity of reference intervals – according to the presented models – is not maintained.

We propose another approach, by which the quality specifications are based on the same principles but with an accurate calculation of reference limits or reference change values. For analytes with a high analytical variation relative to the biological variation, this would result in more realistic quality goals.

This approach is made independent of the criterion by which the test was approved for clinical use. In theory, even a low-quality test with a very high imprecision could be introduced as a routine test by a laboratory. This would result in reference limits and reference change values that are determined predominantly by the analytical variation. In that case, the modified model does still apply.

In the text below, quality specifications based on reference values are presented that apply in the case of diagnosis. It is acknowledged that most tests will be used for monitoring, and the model based on reference change values should be used. Mathematically, this model is very similar to the reference value model. For that reason and for reasons of readability, we refer to Appendix 3: performance specifications for the reference change model.

Table 1: Quality specifications with maximum bias (at $CV_A = 0$) and maximum imprecision (at bias = 0) based on different models for CK and Na.

Model	CK		Na	
	Bias, %	Imprecision, %	Bias, %	Imprecision, %
Conventional (monitoring) [22]	30.3	18.4	0.73	0.44
Gowans (diagnosis)	12.7	27.2	0.25	0.54
Modified (diagnosis)	12.7	27.1	0.39	1.34
Modified (monitoring)	9.0	13.7	0.28	1.30

THE MODIFIED MODEL

This model is an adaptation of Gowans' model (Appendix 1), and the reference change values' model [9, 10] (Appendix 3), based on the following principles:

1. The model describes the maximum bias and imprecision allowable that still maintains the validity of reference values (or reference change values in case of monitoring).
2. The reference limits are defined by both biological and analytical variation.

As in other models, a distinction is made between quality criteria for diagnosis and monitoring. For diagnosis, the CV of the reference value (CV_{ref}) is used as starting point:

$$CV_{ref} = (CV_G^2 + CV_I^2 + CV_{A0}^2)^{1/2},$$

where CV_{A0} is the CV analytical of the test at t = 0, when the reference limits were determined or confirmed.

CV_G = CV(group) and CV_I = CV (within person).

For monitoring, the reference change model is applied (see Appendix 3). This is an adaptation of the model as described before [9, 10]. In both cases, diagnosis and monitoring, the underlying mathematical principles are the same. The only difference is the description of the total variation.

The actual (total) variation of test results in a reference population is based on biological variation and CV_A (CV actual analytical):

$$CV_T(total) = (CV_I^2 + CV_G^2 + CV_A^2)^{1/2}.$$

As in the model of Gowans, a maximum of 4.6% of the test results outside a reference limit is considered acceptable (any other percentage will not change the principle of the model).

The consequence of including CV_{A0} in the expression is that an increase in CV_A with respect to CV_{A0} determines the quality, not the absolute value of CV_A. In this model, CV_{A0} can be within the quality specification $CV_A < 0.5\ CV_I$ but does not need to be, e.g., when the state of the art does not meet this specification. In the model of Gowans, a Gaussian distribution is assumed with CV = CV_B with reference limits at the point where 2.5% of the results are outside the limits. Analytical variation (or analytical variation in combination with bias) is then added to the model with a limit of 4.6% test results outside the reference limits (in other words, 4.6% misclassification instead of 2.5%). In contrast to this, the modified model starts with a Gaussian distribution with $CV = (CV_B^2 + CV_{A0}^2)^{1/2}$ to which additional analytical variation (or analytical variation in combination with bias) is then added.

With $CV_A = 0$, the maximum bias is

$$Bias_{max} = 0.275(CV_B^2 + CV_{A0}^2)^{1/2}.$$

With bias = 0, the maximum imprecision is

$$CV_{A,max} = ((1.96/1.68)^2 \times (CV_B^2 + CV_{A0}^2) - CV_B^2)^{1/2}.$$

The model can be illustrated by the examples in Figures 2 and 3 for CK and sodium.

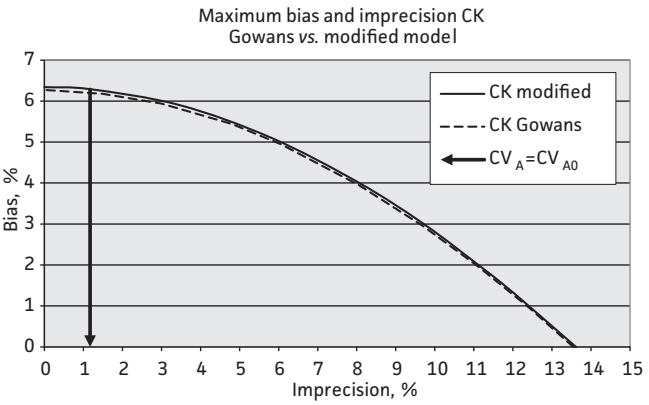

Figure 2: Curves describing the combinations of bias and imprecision for CK according to Gowans' and the modified models.
The arrow indicates the position of CV_{A0} (see Appendix 2). Owing to the low value of the analytical compared to the biological variation, both models are almost identical.

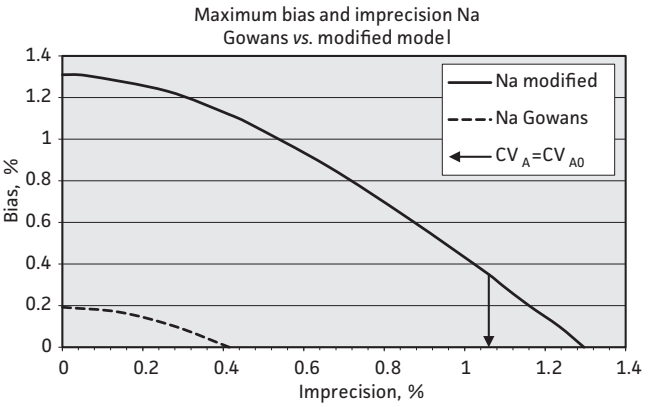

Figure 3: Curves describing the combinations of bias and imprecision for sodium according to Gowans' and the modified models.
Owing to the high value of the analytical compared to the biological variation, both models differ considerably. The arrow indicates the position of CV_{A0} (see Appendix 2). The analytical variation is clearly outside the specifications of Gowans' model but inside the specifications of the modified model.

QUALITY CONTROL, TE_A, AND THE SIGMA CONCEPT

One of the most important uses of quality specifications, in terms of maximum allowable bias and imprecision, is the development of an IQC program. An important concept here is the TE_A. TE_A combines bias and imprecision to one fixed number. This concept is only valid when bias and imprecision show a linear relation (see Appendix 4: linear). The fixed number for TE_A also means

that one does not need to know whether the deviation of a control sample result should be attributed to an increase of bias or imprecision. Only one limit TE_A for the combination of bias and imprecision suffices.

The TE_A concept is valid when IQC results are considered. The distribution of quality control results is described by the analytical variation only, as biological variation plays no role here. For these results, a TE_A limit can be defined for the combination of bias and imprecision; bias and imprecision will show a linear relation as described in Appendix 4.

A problem arises, however, when this concept is translated to patient data. As we have shown, both in the concept of reference values and of reference change values, there is no linear relation between bias and imprecision. When biological variation is taken into account, the linearity changes to a curved relation. This curved relation implies that the tolerance for additional imprecision will increase compared to the tolerance for additional bias, which will remain unchanged. (What might also be taken into consideration is the fact that the sensitivity of multirules [17] are not the same for bias and imprecision.)

Now the problem can be described more precisely: when we have an IQC result with a certain deviation from the target value, we cannot ascribe this to an effect of bias or imprecision. In a linear model, we have shown that this is not of importance, as long as the deviation is within the TE_A limits. As the TE_A concept fails with patient results, with no linear relation between bias and imprecision, how can we decide whether this deviation is acceptable or not?

There is no accepted solution for this problem. We could, however, assume that the imprecision of the test system will remain constant. With that assumption, deviations of IQC results will be ascribed solely to the effect of bias. This pragmatic solution would result in a definition of TE_A for quality control results as

$$TE_A = \text{bias} + Z \times CV_A.$$

With (for diagnosis):

$$Z = 1.65.$$

$$\text{with } CV_A = CV_{AO} \text{ (see Appendix 2).}$$

$$CV_{TO} = (CV_I^2 + CV_G^2 + CV_{AO}^2)^{1/2}.$$

$$\text{Bias}_{max} = -1.68CV_{TO} + 1.96CV_{TO} = 0.275CV_{TO}.$$

$$TE_A = 0.275CV_{TO} + 1.65CV_{AO}.$$

For monitoring, see Appendix 3: modified RCV.

A test can have a certain analytical quality that is close to the limit of the quality specifications. In that case, there will be a problem with the maintenance of this level of quality. For instance, when $CV_A = 0.5CV_B$ (see Figure 1), the analytical variation is almost equal to the limit of the desired quality specification according to the criteria based on biological variation [23]. In Gowans' model, no additional bias or imprecision is allowed. This makes it almost impossible to apply quality control specifications to the test, even with the quality specification fulfilled. The modified model, however, has a considerable margin for additional bias and imprecision in the case of CK (Figure 2, vertical line), and some margin for sodium (Figure 3).

In conclusion, some extra margin of quality is needed because of the limited sensitivity of ICQ procedures. Note that within the Six Sigma theory, a margin of 1.5 SD is assumed necessary to maintain the results within the performance specifications.

DISCUSSION

In this study, we compared several models for analytical quality specifications, including a modified model based on the calculation of reference values and reference change values, taking the analytical variation into account. Central to this model is the assumption that the validity of reference limits (for diagnosis) or reference change values (for monitoring) determines the minimum analytical quality. This concept can, of course, in itself be discussed.

A distinction is made with, on the one hand, the analytical performance needed for routine clinical use of a test, answering the question of whether a test should be taken into routine use by the laboratory. On the other hand is the analytical performance required to maintain the validity of the reference limits and reference change values, once the choice has been made to take a test into use. The second follows the first: when a test is accepted for routine use based on clinical or other criteria, reference limits are subsequently determined (or existing reference limits validated). Quality criteria can subsequently be derived from the model that maintains the validity of the reference limits or reference change values.

Both models of Klee and Gowans [8, 16] are based on the reference value concept and do include the biological variation, but do not include analytical variation in their definition of reference limits. This can lead to unrealistic values and quality criteria for tests like sodium, where the analytical variation is the dominant component of the reference interval.

The calculation of TE_A is often based on biological variation. It has been shown that in the calculation of TE_A, the summation of both maximum allowable bias and imprecision term [expression (2)] does lack a theoretical basis [7]. In the conventional model, the total allowable error is assumed to be a constant, with an inherent linear relation between bias and imprecision. The biological variation is, however, not correctly included in this model. In the presented model, TE_A – with analytical variation included – is not anymore a constant but depends on the ratio of bias and analytical variation.

The proposed modified model can be seen as an adaptation of existing models based on reference values [8] and reference change values [9, 10]. In these models, reference limits were calculated based on biological variation alone. In the present model, reference limits are based on both biological and analytical variation. In tests with CV_B considerably larger than CV_A, CV_A can, however, be neglected in the calculation, and the model equals to the existing models. When this condition is not fulfilled, the quality goals according to the existing models will tend to be too strict. For example, for sodium, it would be almost impossible to meet the quality specifications (Figure 2). In contrast, in the modified model, both bias and imprecision do meet the quality specifications and have still some margin for increase. In the case of a less-than-perfect test, application of the modified model will lead to more realistic quality goals.

In the model of Gowans, the quality specification for misclassification was 4.6%. In the presented model, we applied this specification of 4.6% both for the case of diagnosis and for monitoring, although in the model of Gowans this specification has been derived for the validity of reference values (and thus for diagnosis) only. Another specification could, however, be applied, without changing the principles of the presented model.

On the one hand, we have quality specifications based on the validity of reference values and reference change values. On the other hand, we have the problem of maintaining this quality with quality control procedures. These procedures have a limited sensitivity for errors, and an extra quality margin is needed to be able to guarantee that results are within quality limits. This margin will depend on the quality control procedures applied, and is not part of the models presented here.

CONCLUSIONS

We propose a modified model that offers an alternative method for the calculation of performance specifications. It is based on maintaining the validity of reference limits and reference change values. The model applies to almost any test if biological variation can be estimated. A pragmatic method for the design of IQC is presented.

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NOTES

Note 1

Z-value 1.65 *vs.* 1.96.

When the bias is equal or larger than the analytical imprecision, the Z-value of 1.65 applies, and 95% of the test results will fall within the limits defined by the TE_A. The test result can be reported with 95% certainty to be within the limit defined by this TE_A.

The Z-factor has been shown to be dependent on the ratio of bias and imprecision and could be 1.96 (twosided 0.95 or 95% confidence limit) when bias can be neglected compared to the imprecision. For ratios of bias and imprecision, the Z-value was calculated as 1.96 (bias/imprecision = 0), 1.77 (ratio = 0.25), 1.68 (ratio = 0.5), 1.65 (ratio = 0.75), and 1.645 (ratio ≥ 1) [24].

For example, when the true value of glucose is 9 mmol/L with a bias of 6% and an imprecision of 2% (ratio > 1), then the bias is 9 mmol/L × 6/100 or 0.54 mmol/L and the imprecision 9 mmol/L × 2/100 or 0.18 mmol/L. This makes TE 0.54 + 1.645 × 0.18 or 0.84 mmol/L greater than the actual concentration of 9.0 mmol/L, so that the TE limit is 9.84 mmol/L.

In this case, the bias is positive and substantially greater than the imprecision. The Z-value is 1.645 in this case, as only the upper limit is relevant: knowing with 95% certainty that the true glucose concentration will not exceed the 9.84 mmol/L limit. With this bias, the measured value will never be lower than the true value.

If, on the other hand, the bias is low, the Z-value of 1.96 should be applied. For example, when the true value of glucose is 9 mmol/L with a bias of 0% and an imprecision of 2%, then Z is 1.96. The imprecision component is 9 mmol/L × 1.96 × 2/100 or 0.35 mmol/L. This makes the TE 0.0 + 0.35 or 0.35 mmol/L higher or lower than the actual concentration of 9.0 mmol/L so that the TE limits are 8.65 and 9.35 mmol/L.

As a known bias can be corrected, the presence of a bias that is equal or larger than the imprecision of a test should, in many cases, be corrected. As a result, use of the Z-value of 1.65 in all cases could be questioned.

Note 2

Gowans' model allows a maximum increase of 84%: (4.6–2.5)/2.5 × 100% = 84%.

Note 3

Note that these results are in close agreement with the accepted performance criteria [25]: for bias, compare $0.275CV_B$ (CV biological) with the maximum (desirable) bias of $0.25CV_B$.

For imprecision, compare $0.597CV_B$ with $0.5CV_I$. Note that Gowans' model only mentioned biological variation and did not take into account the difference between diagnosis (based on within group and within person variation) and monitoring (based only on within-person variation).

APPENDIX

Appendix 1. Model of Gowans et al. (Gowans)

When the recommendations of the IFCC are applied, reference values will be calculated on the basis of test results in a group of at least 120 persons [21]. There is always an inherent uncertainty in the determination of the reference limits for every analyte. This uncertainty is such that with n = 120, a maximum of 4.6% (one-sided) of the results could be outside the "inner" confidence limit of the reference limits when using 1.96 SD.

Following this model, a curve can be calculated that defines the maximum bias and imprecision with 4.6% of results outside the reference limit. Maximum bias and imprecision are at the extreme ends of this curve are (see Figure 1).

In general, the relation between bias and imprecision can, in this case, be described as (see Appendix 4)

$$\text{Bias} = -1.68CV_T + 1.96CV_B,$$

with CV_T = total variation = $(CV_B^2 + CV_A^2)^{1/2}$; CV_B = total biological variation; CV_A = analytical variation; 1.96 represents the Z-value with 2.5% and 1.68 the Z-value with 4.6% outside the limit.

In this expression, bias and imprecision (analytical variation) have a linear relationship with a slope of –1.68 (see Appendix 4). The intersection with the y-axis represents the bias at $CV_A = 0$:

$$\begin{aligned}\text{Bias}(CV_A = 0) &= -1.68(CV_B^2 + CV_A^2)^{1/2} + 1.96CV_B \\ &= -1.68(CV_B^2)^{1/2} + 1.96CV_B \\ &= (1.96 - 1.68)CV_B.\end{aligned}$$

Maximum bias (when imprecision = 0) = $0.275CV_B$.

Note that this number differs from 0.25 in the study of Gowans et al. [8].

The intersection with the x-axis represents CV_A at bias = 0

$$CV_A(\text{bias} = 0) : 0 = -1.68(CV_B^2 + CV_A^2)^{1/2} + 1.96CV_B.$$

$$1.96CV_B = 1.68(CV_B^2 + CV_A^2)^{1/2}.$$

Maximum imprecision (when bias = 0) = $0.597CV_B$.

Between these two extremes, a curve describes the combination of bias and imprecision such that the condition is fulfilled: 4.6% of the results outside the reference limits (one-sided) (Figure 1) (Note 3).

Calculation of maximum bias and imprecision of CK and sodium:

Example 1: CK

Gowans' model, with maximum imprecision and bias, respectively:

$$CV_{A,max} = 0.597CV_B.$$

$$\text{Bias}_{max} = 0.275CV_B.$$

$$CV_I = 22.8\%;\ CV_G = 40.0\%\ [22].$$

$$CV_B = (CV_I^2 + CV_G^2)^{1/2} = 46.0\%.$$

$$CV_{A,max} = 0.597CV_B = 27.2\%.$$

$$\text{Bias}_{max} = 0.275CV_B = 12.65\%.$$

Example 2: sodium

$$CV_I = 0.6\%;\ CV_G = 0.7\%\ [22].$$

$$CV_B = (CV_I^2 + CV_G^2)^{1/2} = 0.92\%.$$

$$CV_{A,max} = 0.597CV_B = 0.54\%.$$

$$\text{Bias}_{max} : 0.275CV_B = 0.25\%.$$

Note that these quality limits apply for diagnosis, not for monitoring situations.

Appendix 2. Modified model, adapted Gowans' model

Below is the modification of Gowans' model, based on the following principles:

1. The model describes the maximum bias and imprecision allowable to maintain the validity of reference values (or reference change values in case of monitoring).
2. The reference limits are defined by both biological and analytical variation.

As in other models, a distinction is made between quality criteria for diagnosis and monitoring. For diagnosis, the CV of the reference value (CV_{ref}) is used as starting point:

$$CV_{ref} = (CV_G^2 + CV_I^2 + CV_{AO}^2)^{1/2},$$

with CV_{A0} = CV analytical of the test at t = 0, when the reference limits were determined or confirmed; CV_A = CV actual analytical.

For monitoring, the reference change model is applied (see Appendix 3). This is an adaptation of the model as described before [9, 10]. In both cases, diagnosis and monitoring, the general principles presented here are the same.

The actual (total) variation CV_T of test results in a reference population is based on biological variation and actual analytical variation CV_A:

$$CV_T = (CV_I^2 + CV_G^2 + CV_A^2)^{1/2}.$$

As in the model of Gowans, a maximum of 4.6% of the test results outside a reference limit is considered acceptable (any other percentage is, however, possible and will not change the principle of the model). The curve in this modified model is identical to the curve according to Gowans, with the following substitutions.

The relation between bias and imprecision can, in this case, be described as (see Appendix 4)

$$\begin{aligned}\text{Bias} &= -1.68(CV_B^2 + CV_A^2)^{1/2} + 1.96(CV_B^2 + CV_{AO}^2)^{1/2} \\ &= -1.68(CV_I^2 + CV_G^2 + CV_A^2)^{1/2} + 1.96(CV_G^2 + CV_I^2 + CV_{AO}^2)^{1/2}.\end{aligned}$$

$$CV_B = (CV_I^2 + CV_G^2)^{1/2},$$

where 1.96 represents the Z-value with 2.5% and 1.68 the Z-value with 4.6% outside the limit, respectively.

The consequence of including CV_{A0} in the expression is that an *increase* in CV_A with respect to CV_{A0} determines the quality, not the absolute value of CV_A.

In the model of Gowans, a Gaussian distribution is assumed with CV = CV_B with reference limits at the point where 2.5% of the results are outside the limits. Analytical variation (or analytical variation in combination with bias) is then added to the model with a maximum of 4.6% test results outside the reference limits. In contrast to this, the modified model starts with a Gaussian distribution with CV = $(CV_B^2 + CV_{A0}^2)^{1/2}$ to which analytical variation (or analytical variation in combination with bias) is then added.

With bias = 0, the maximum allowable imprecision should fulfill the condition

$$\text{Bias} = 0 = -1.68(CV_B^2 + CV_{A,max}^2)^{1/2} + 1.96(CV_B^2 + CV_{AO}^2)^{1/2}.$$

The maximum CV_A can be calculated from this expression to be

$$CV_{A,max} = ((1.96 / 1.68)^2 \times (CV_B^2 + CV_{AO}^2) - CV_B^2)^{1/2}.$$

With $CV_A = 0$, the maximum bias is

$$\text{Bias}_{max} = 0.275(CV_B^2 + CV_{AO}^2)^{1/2}.$$

The model can be illustrated by the examples in Figures 1 and 2.

Example 1: CK

In the case that $CV_{A0} < < CV_B$, the term CV_{A0} vanishes from the expression and the modified model equals Gowans' model (Figure 1), with maximum imprecision and bias, respectively

$$CV_{A,max} = 0.597CV_B.$$

$$\text{Bias}_{max} = 0.275CV_B.$$

Note that, in this case, the modified model will become equal to Gowans' model.

CK approximates this condition with $CV_{A0} << CV_B$. Numbers from the authors' laboratory:

$$CV_{A0} = 1.17\%.$$

$$CV_I = 22.8\%;\ CV_G = 40.0\%\ [22].$$

The contribution to the reference interval by the analytical variation is only 0.03%.

Note:

$$CV_I = 22.8\%;\ CV_G = 40.0\%.$$

$$(CV_I^2 + CV_G^2 + CV_{AO}^2)^{1/2} = (22.8^2 + 40.0^2 + 1.17^2)^{1/2} = 46.01\%.$$

$$(46.01 - 46.0) / 46 \times 100\% = 0.03\%.$$

$CV_{A,max}$ according to the modified model:

$$(CV_{biol}^2 + CV_{A,max}^2)^{1/2} = 1.16(CV_{biol}^2 + CV_{AO}^2)^{1/2} \rightarrow CV_{A,max} = 27.1\%.$$

$$\text{Bias}_{max} = 0.275(CV_{biol}^2 + CV_{AO}^2)^{1/2} = 12.65\%.$$

This illustrates that for $CV_{A0} << CV_B$, both models become equal.

Example 2

On the other end of the spectrum, we have $CV_{A0} >> CV_B$, and now the term CV_B vanishes from the expression. Gowans' model does not apply to this situation, as CV_{A0} lies outside the area of the curve (outside the minimum quality limit).

Sodium approximates this condition with $CV_{A0} > CV_B$. Numbers from the authors' laboratory (see Figure 2):

$$CV_{A0} = 1.06\%.$$

$$CV_I = 0.6\%;\ CV_G = 0.7\%\ [22];\ CV_B = 0.92\%.$$

The contribution to the reference interval by the biological variation is 17%.

$CV_{A,max}$, modified model:

$$(CV_B^2 + CV_{A,max}^2)^{1/2} = 1.16(CV_B^2 + CV_{AO}^2)^{1/2} \rightarrow CV_{A,max} = 1.34\%.$$

$$\text{Bias}_{max} = 0.275(CV_B^2 + CV_{AO}^2)^{1/2} = 0.39\%.$$

Appendix 3. Performance specification based on reference change values

Modified model

Below is the adaptation of the model based on reference change value [9, 10], according to the following principles:

1. The model describes the maximum bias and imprecision necessary to maintain the validity of reference change values.
2. The reference change values are defined by both biological and analytical variation.
3. By definition, reference change values only apply for monitoring, not for diagnosis.

The reference change value (RCV) is defined as

$$RCV = \sqrt{2} \times Z \times (CV_I^2 + CV_A^2)^{1/2},$$

CV_A = CV analytical,

CV_I = CV within person,

where Z is the number of standard deviations appropriate to the desired probability.

The differences between two consecutive values within one patient are described by a Gaussian curve with CV:

$$CV_{RCV} = \sqrt{2}(CV_I^2 + CV_A^2)^{1/2}.$$

With a Z-value of 1.96, 2.5% of the test results will respectively be outside the upper and lower limits. In analogy with the quality limits described before, we chose to set the quality standard at a maximum of 4.6% outside upper and lower limits, instead of 2.5%. In other words, bias and imprecision – or the combination of these – are allowed to increase until 4.6% of the differences (change values) of a reference population are outside a reference limit, resulting in a 4.6% misclassification instead of 2.5%.

Again, we substitute CV_{A0} (CV_A at the time the test was introduced or validated) for CV_A. The consequence of this is that it is the *increase* in CV_A with respect to CV_{A0} that determines the quality, not the absolute value of CV_A.

$$RCV = \sqrt{2} \times Z \times (CV_I^2 + CV_{AO}^2)^{1/2}.$$

The combinations of maximum bias and imprecision are again described with a curve.

With imprecision = CV_{A0}, there is no additional imprecision compared to CV_{T0}, and the maximum bias can be calculated (a decrease of imprecision relative to CV_{T0} is possible but not considered here):

$$Bias = 1.96 \times \sqrt{2}CV_{TO} - 1.68 \times \sqrt{2}CV_T.$$

Note: 1.96 is Z-value corresponding with 2.5%; 1.68, with 4.6% outside the quality limit.

With $CV_{TO} = (CV_I^2 + CV_{AO}^2)^{1/2}$,

$$CV_T = (CV_I^2 + CV_A^2)^{1/2}.$$

Maximum bias is allowed when $CV_A = CV_{A0}$, and $CV_T = CV_{T0}$

$$Bias_{max} = (1.96 - 1.68) \times \sqrt{2}CV_{TO},$$

or

$$Bias_{max} = 0.275 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2}.$$

With bias = 0, the maximum allowable imprecision should fulfill the condition:

$$Bias = 0 = 1.96 \times \sqrt{2}CV_{TO} - 1.68 \times \sqrt{2}CV_T,$$

With $CV_{T0} = (CV_I^2 + CV_{AO}^2)^{1/2}$,

And $CV_T = \sqrt{2}(CV_I^2 + CV_A^2)^{1/2}$,

$$Bias = 0 = 1.96 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2} - 1.68 \times \sqrt{2}(CV_I^2 + CV_A^2)^{1/2}.$$

The maximum CV_A can be calculated from this expression to be

$$CV_{A,max} = ((1.96 / 1.68)^2 \times (CV_I^2 + CV_{AO}^2) - CV_I^2)^{1/2}.$$

Example 1

CK approximates this condition with $CV_{A0} < < CV_B$. Numbers from the authors' laboratory:

CV_{A0} = 1.17%.

CV_I = 22.8% [22].

$CV_{A,max}$ according to the reference change model:

$$CV_{A,max} = ((1.96 / 1.68)^2 \times (CV_I^2 + CV_{AO}^2) - CV_I^2)^{1/2} = 13.7\%.$$

Maximum bias:

$$Bias_{max} = 0.275 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2} = 8.97\%.$$

Example 2

Sodium approximates the condition with $CV_{A0} > CV_B$. Numbers from the authors' laboratory (see Figure 2):

CV_{A0} = 1.06%.

CV_I = 0.7% [22].

$CV_{A,max}$ according to the reference change model:

$$CV_{A,max} = ((1.96 / 1.68)^2 \times (CV_I^2 + CV_{AO}^2) - CV_I^2)^{1/2} = 1.3\%.$$

Maximum bias:

$$Bias_{max} = 0.275 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2} = 0.28\%.$$

Estimated TE_A for monitoring

$$TE_A = bias + Z \times CV_A,$$

with (for monitoring)

$$Z = 1.65.$$

$$CV_A = CV_{A0}.$$

$$CV_{TO} = (CV_I^2 + CV_G^2 + CV_{AO}^2)^{1/2}.$$

$$Bias = -1.68 \times \sqrt{2}CV_{TO} + 1.96 \times \sqrt{2}CV_{TO} = 0.275 \times \sqrt{2}CV_{TO} = 0.39CV_{TO}.$$

$$TE_A = 0.39CV_{TO} + 1.65CV_{AO}.$$

Appendix 4. Linear relation between bias and imprecision

Assume that test results show a normal (Gaussian) distribution with coefficient of variation CV_{T0} (total coefficient of variation at t = 0, biological and analytical combined). We can define a limit (e.g., a quality limit) with a fixed number (percentage) of test results outside this predefined limit. The position of this

limit is expressed as Z-factor (number of CV). When the percentage of test results outside the limit is 2.5% (one-sided), Z equals 1.96.

If the bias (shift of the curve) increases from zero to a positive number, the percentage of test results outside the limit +1.96 × CV_{T0} will increase, above the predefined number of 2.5%. To fulfill the condition of 2.5%, CV_T (actual total coefficient of variation) should decrease. The relation between bias and CV_T and CV_{T0} can be expressed as

$$\text{Bias} = -Z \times CV_T + Z \times CV_{TO}.$$

In the case of zero bias, the (maximum) CV will be equal to CV_B, and the condition of 2.5% outside the predefined limit is fulfilled.

In the case of zero CV_T, the (maximum) bias will be equal to Z × CV_{T0}, a shift equal to the position of the predefined limit. (Strictly speaking, CV_T = 0 outside the model, as this CV_T does not define a normal distribution. However, as CV approaches zero, the bias approaches the limit Z × CV_B.)

In the case of CV_T half of CV_{T0}, the (maximum) bias will be equal to 0.5 × Z × CV_{T0}, or a shift of 0.98CV_{T0}.

There is another situation if the distribution of results is defined by CV_{T0}, the limit at Z = 1.96 with 2.5% of the results outside the predefined limit. A new maximum percentage can be set at 4.6%, at the same limit of 1.96CV_{T0}; 4.6% corresponds to a limit at 1.68CV_{T0}, so the corresponding Z-value is 1.68. The relation between bias and CV_T can now be expressed as

$$\text{Bias} = -Z'CV_T + Z \times CV_{TO} = -1.68CV_T + 1.96CV_{TO}.$$

In the case of zero bias, the (maximum) CV_T will be equal to Z/Z´ × CV_{T0}, or (1.96/1.68) × CV_{T0}, and the condition of 4.6% outside the predefined limit is fulfilled.

In the case of zero CV_T, the (maximum) bias will be equal to Z × CV_{T0} (1.96CV_{T0}), a shift equal to the position of the predefined limit. The linear relation between bias and CV only exists as the distribution is described by CV. The total variation can be composed of components of biological and analytical variation. The relation between a component (e.g., the analytical variation) and bias will not be linear, but is described by a curve (see Figure 1).

Appendix 5. Definition of pragmatic quality control limits

Here, it is assumed that the deviation of IQC results is mainly due to bias, and that the increase of the analytical variation can be neglected. For diagnosis:

$$TE_A = \text{bias} + Z \times CV_A, \quad (1)$$

with

$$Z = 1.65. \quad (2)$$

We assume that the actual CV_A is equal to the CV_A at time = 0 (stable CV_A):

$$CV_A = CV_{A0}. \quad (3)$$

For diagnosis, the total variation at t = 0:

$$CV_{TO} = (CV_I^2 + CV_G^2 + CV_{AO}^2)^{1/2}. \quad (4)$$

For bias (see Appendix 4: linear):

$$\text{Bias} = -1.68(CV_I^2 + CV_G^2 + CV_A^2)^{1/2} + 1.96(CV_G^2 + CV_I^2 + CV_{AO}^2)^{1/2}. \quad (5)$$

With $CV_A = CV_{A0}$ [5], this becomes

$$\text{Bias} = -1.68CV_{TO} + 1.96CV_{TO} = 0.275CV_{TO}. \quad (6)$$

Expression (1) combined with (2), (3), and (6) becomes

$$TE_A = 0.275 \times CV_{TO} + 1.65CV_{TO}. \quad (7)$$

Compare this with the expression for monitoring (Appendix 3: RCV):

$$\text{Bias} = 1.96 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2} - 1.68 \times \sqrt{2}(CV_I^2 + CV_A^2)^{1/2}. \quad (8)$$

With $CV_A = CV_{A0}$ [5], this becomes

$$\text{Bias} = 0.275 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2}. \quad (9)$$

Expression (1) combined with (2), (3), and (6) becomes

$$TE_A = 0.275 \times \sqrt{2}(CV_I^2 + CV_{AO}^2)^{1/2} + 1.65CV_{AO}.$$

$$TE_A = 0.39(CV_I^2 + CV_{AO}^2)^{1/2} + 1.65CV_{AO}. \quad (10)$$

For CK, this will give a sigma score (numbers as used before):

With CV_{A0} = 1.17%; CV_I = 22.8%.

TE_A = 10.8%.

Sigma score (with $CV_A = CV_{A0}$) : TE_A/CV_{A0} = 9.3.

For sodium:

With CV_{A0} = 1.06%; CV_I = 0.6%.

TE_A = 2.2%.

Sigma score (with $CV_A = CV_{A0}$) : TE_A/CV_{A0} = 2.1.

It can be calculated that for a sigma score of 3.0, CV_A (with $CV_A = CV_{A0}$) should be 0.18%.

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PL-3. Laboratory Medicine in Europe

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The profession of Laboratory Medicine differs between countries within the European Union (EU) in many respects. The objective of professional organizations for promotion of mutual recognition of specialists within the EU is closely related to the free movement of people. This policy translates to equivalence of standards and harmonization of the training curriculum. In a study that was supported by both the Union Européenne de Médecins Spécialistes (UEMS) and European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), the organization and practice of Laboratory Medicine was evaluated within the countries that constitute the EU. A questionnaire covering many aspects of the profession was sent to delegates of the EFLM and UEMS of the 28 EU countries. The results were sent to the delegates for confirmation. Many differences between the countries were identified: predominantly medical or scientific professionals; broad or limited professional field of interest; inclusion of patient treatment; formal or absent recognition; regulated or absent formal training programme; general or minor application of a quality system based on ISO Norms. Harmonization of the postgraduate training of both clinical chemists and laboratory physicians has been a goal for many years. Differences in the organization of the laboratory professions still exist in the respective countries which all have a long historical development with their own rationality. It is an important challenge to harmonize our profession, while difficult choices should be made. Recent developments with respect to the directive on Recognition of Professional Qualifications call for new initiatives to harmonize Laboratory Medicine both across national borders, and across the borders of scientific and medical professions.

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SESSION 1

OP-1. Improving the efficiency of clinical laboratory services

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Automation of laboratory analyses over the past 50 years has greatly increased efficiency in terms of the speed with which analytical results become available but speed is not the only factor contributing to efficiency. It is also important to maintain quality. More recently, the extension of automation to both the pre- and post-analytical phases has not only improved turnaround time but reduced opportunities for human error.

However, analysis is not the only process to be considered. By examining overall workflow, using management techniques such as LEAN principles, wasteful steps can be eliminated and processes streamlined. Efficiency is improved by standardised practice, has developed to ensure quality and hence reduce errors. Use of such management techniques is dependent on teamwork and a committed, highly trained workforce.

Improvement of efficiency should not be considered in isolation. It is essential that service users are involved in defining the level of service expected as well as in defining what is required for appropriate patient care. Also, collaboration with other laboratories, for example in terms of sharing instrumentation or in decisions on which tests to offer on site, may provide further opportunities for more effective and efficient provision of service.

OP-2. Laboratory management through consolidation / automation

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Consolidation and automation are a central issue in simplifying the laboratory process. We will show, using some examples, how automation has developed step by step. The next breakthrough is consolidation. It leads to complex work areas, speeds up the workflow and changes the job content of all professional categories in the laboratory. High throughput, support by information systems and consolidated work areas are characteristic of an up-to-date laboratory. We will also present some facts and calculations showing how parameters like turn-around-time are affected by these changes.

LIS: the basics

Following our understanding of the laboratory process, we will show how single steps in this process can be supported by a LIS. We start with the request and the different ways to communicate between requester and laboratory. Next we will present some examples of how the steps of the process within the laboratory can be supported. At last we will demonstrate different ways how to bring the results from the laboratory to the clinician.

ORAL PRESENTATIONS

SESSION 1

OP-3. Laboratory consolidation: a Finnish example of networking

Ari Miettinen – Fimlab Laboratories, Tampere, Finland

Over the last two decades technological innovations have created the potential for substantially improving the productivity of clinical laboratories. In many countries, however, the organization and business model of laboratories suffer for fragmentation, redundancy, and low capacity utilization. The monopoly position of many hospital laboratories as well as generous reimbursement policies may have allowed laboratories to become stuck with traditional specialty-defined models of organization and production. Hence, technological advancements have not brought about cost savings to the degree that might have been anticipated.

In the early 2000s the pressure to reduce healthcare costs focused the attention of the Finnish government on the way the country's diagnostic services were organized. A government initiative was proposed to the public healthcare authorities with a view to reorganizing diagnostic services throughout the country in accordance with the consolidation and networking process of Fimlab Laboratories in its area. Moreover, a cut was made in reimbursements in order to boost a similar development in the private sector.

Within 15 years the number of laboratories within the public sector has decreased from more than 400 independent laboratory units to 11 regional network organizations. The five largest of these cover about 4.5 million (85%) of Finland's total population of 5.3 million people. A similar consolidation process through acquisitions and networking has taken place among the private clinics and their laboratories.

As a consequence of consolidation and networking, the productivity of the laboratory branch has increased dramatically compared to that of the Finnish healthcare sector in general. Additional value has been created through harmonization and the availability of test results within the networks. An endpoint to the consolidation process is not yet in sight.

OP-4. Quality indicators in laboratory medicine: from theory to practice

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Identification and measurement of quality indicators (QIs) in the whole testing process (TTP) are obligatory requirements for accreditation of laboratories. A model of quality indicators (MQI) has been consensually developed by a group of clinical laboratories on the basis of a project launched by the Working Group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The model includes 27 QI related to key processes (12 pre-, 5 intra- and 5 post-analytical phases) and 5 to support and outcome processes. The QI are divided, according to priority, into 4 classes: mandatory, important, suggested and valued. It is expected that this approach will be used in all kind of medical laboratories thus focusing on patient safety.

SESSION 2

OP-5. Monitoring new oral anticoagulants

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Direct oral anticoagulants (DOACs), the thrombin inhibitor dabigatran and the factor Xa (FXa) inhibitors rivaroxaban, apixaban and edoxaban are in clinical use in many countries. Clinical trials confirm that DOACs may be used for prevention of stroke in atrial fibrillation and for prevention and treatment of venous thromboembolism effectively and safely, without dose adjustment based on laboratory testing. The predictable pharmacodynamics and kinetics of DOACs make routine coagulation testing unnecessary. However, there are circumstances in which laboratory measurements are indicated and even vital. In case of acute hemorrhage or thrombosis, emergency surgery, reversal of anticoagulation, or renal dysfunction, clinicians may desire to determine the presence or concentration of a drug.

Numerous laboratory assays may reveal the effects of DOACs. The widely available routine coagulation assays INR, PT and APTT, lack sensitivity and are inadequate for drug monitoring. A normal APTT most likely excludes excess dabigatran levels, and normal thrombin time (TT) excludes clinically relevant levels. Dilute-TT and ecarin-based assays can quantify dabigatran. The FXa inhibitors may be quantified with an anti-FXa assay calibrated with drug-specific standards. Among others, Russel's Viper Venom Time and thrombin generation assays yield interesting findings for research. On the contrary, DOACs impact specialty coagulation assays potentially causing *in vitro* false results.

Limitations of coagulation testing should be noted. Initially, most data are based on drug-spiked plasma studies rather than on samples from real-life patients receiving DOAC therapy in clinical settings. Inter-individual variation is obvious, and coagulation methods are reagent- and laboratory-dependent. Thus, available data must be interpreted with caution and more data is needed in order to establish alert values for clinicians. Laboratory could also guide the economical use of new reversal agents. These new settings of anticoagulation treatment represent a challenge for clinical laboratories.

OP-6. Permanent fight between Owren and Quick

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The story of Owren and Quick began early in the last century. Back in 1935 Armand James Quick (1894–1978) developed a prothrombin time (PT) assay, where excess of thromboplastin and calcium is added to anticoagulated plasma. Later, in 1959 Paul Arnor Owren (1905–1990) created a modified PT version. His main objective was to overcome the drawbacks of the Quick method. Since then the two PT methods has been competing. Each has its own supporters, Quick having almost 90 percent of the total. Nevertheless, minor popularity of Owren does not mean that it is worse. Thus the aim of the speech is to provide the cons and pros of both methods.

There are three basic technical differences between Owren and Quick PTs. First, Quick PT measures coagulation factors FI, FII, FV, FVII and FX, and Owren PT measures only FII, FVII and FX. Consequently, Quick has an advantage in the diagnosis of coagulation factor deficiencies, since it measures the coagulation factor FV. However, it is a drawback in oral anticoagulation treatment (OAT) follow-up, since OAT medication does not affect FV synthesis. Second, test mixtures differ in the amount of constituents (Quick: sample 50 µl + reagent 100 µl; Owren: sample 10 µl + diluent 50 µl + reagent 140 µl). Quick reagent`s volume is 40% smaller which means lower expenses per test. Third, sample volumes in a reaction mixture are different (Quick's – 33%; Owren's – 5%). This means that the Quick method is more sensitive to pre-analytical variables such as heparin, citrate concentration and some other drugs.

Literature provides data that agreement between Owren and Quick PT's is poor. This might compromise not only monitoring of individual patients, but also application of oral anticoagulation guidelines and trial results in clinical practice. Some authors have shown that Owren PT is much more consistent in a OAT setting, and have hence postulated a quite challenging question: "Has time arrived to replace the Quick PT test for monitoring OAT?" The answer cannot be straightforward.

SESSION 2

OP-7. Screening for hemoglobinopathies

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Hemoglobinopathy conditions consist of a variety of the hemoglobin disorders caused by structural abnormalities and/or alteration in the amount of globin chains. Thalassemia is the most common type of hemoglobinopathy transmitted by heredity. Non-endemic countries such as Estonia, are also involved in thalassemia-related problems because migration from endemic to non-endemic areas is bound to increase. In non-endemic countries physicians need to know how to diagnose thalassemias and how to distinguish them from other causes of a microcytic anemia, as well as to have knowledge of treatment options for severe forms of thalassemia.

The basic strategy for screening of hemoglobinopathies requires integration of:

- Complete blood count, reticulocytes
- Morphology of peripheral blood smear
- Iron status assays: iron, ferritin, transferrin saturation, serum soluble transferrin receptor. Transferrin and ferritin are acute phase reactants and values may change in iron deficiency with coexistent inflammation or liver disease. It is best to measure them at the time when no acute illness is present.
- Hemolysis parameters: bilirubin, LDH, haptoglobin (acute phase protein)
- Hemoglobin electrophoresis

Hemoglobin electrophoresis should be done to all patients with microcytic anemia who are not iron deficient or who do not respond to iron replacement therapy.

The reason for referral and information on the clinical history and medication are required for the interpretation of results.

Screening for hemoglobinopathies is very important in non-endemic countries and requires focused attention. DNA analysis will confirm the nature of any haemoglobin variant.

OP-8. Relationship between clinicians and specialists of laboratory medicine in diagnostics of haemostasis disorders

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Clinical and laboratory diagnostics have become more complicated compared to the diagnostics of the past. Haemostasis laboratories (as well as other laboratories) have introduced a number of new tests and modern technologies during the past decades.

Increase of diagnostic possibilities gives physicians more chance to make a wrong choice – to order inappropriate lab tests, to miss informative tests or to make wrong interpretations of correctly ordered tests. Undetected or ignored presence of preanalytical factors (omission of sampling requirements, unsuitable samples, ignorance or missing information about anticoagulant therapy, etc.) causes changes of lab test results. Spurious results in routine coagulation and in specialized haemostasis testing could also lead to serious adverse clinical outcomes and undesirable economic consequences.

Laboratory specialists quite often concentrate their efforts on technical performance and on achievement of the highest quality of tests. They often tend to forget about the conversion of lab data into useful information for clinicians and for the patients.

Patient-centered outcome and economy-related problems may be solved by two sided communication between clinicians and lab professionals. Communication could help to diagnose haemostasis disorders more quickly and accurately, in cases with less than optimal combination of tests for improving the situation, to eliminate unnecessary haemostasis tests, to ensure feedback to physicians about their erroneous ordering practices, to provide information about new or less known tests, etc.

It has been reported that adverse clinical outcomes due to errors (or lack) in communication are more frequent compared to errors due to inadequate clinical skills. To identify the causes of poor communication and to find ways for improvement are consequently of crucial importance. In order to increase the benefit of laboratory testing for diagnostics, laboratory specialists should focus on active development of relationship with clinicians.

SESSION 3

OP-9. Critical risk results in Europe

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Management of critical risk results (CRR), i.e. laboratory results that require immediate medical attention and action because they indicate a high risk of imminent death or major patient harm, is considered a significant patient safety factor.

The Task and Finish Group on Critical Results (TFG-CR) of the European Federation of Clinical Chemistry and Laboratory Medicine collected information on the practice of 871 European laboratories in CRR management and analysed the ALs of almost 400 laboratories within the frames of an international survey in 2012. This presentation will discuss the observed CRR management practices in the light of the recommendations of the recently published standard in the field of CRR management (CLSI-GP47).

The fundamental requirement of CLSI-GP47 on the necessity of organisational-specific ALs and CRR reporting policy was found to be satisfied in a vast majority of the participating laboratories. However, significant variations were seen between the laboratories in the aspects regarding how they compiled their alert lists (ALs) as well as regarding which tests these laboratories selected for inclusion in ALs. The most important nonconformities in the designing of ALs were the following: the thresholds were often not set on broad consensus at institutions; less than half of the laboratories reported to have set timeframes in reporting; and only 16% of the provided ALs contained indication of the different urgencies of notification linked to certain values. Wide variations were also observed in the reporting process of CRRs among the laboratories. Their policies were not always based on the patients` clinical condition. Only a third of the respondents had established practices for proper documentation or monitoring of the performance of their CRR notifications.

The findings of the European survey confirm the need for organising training courses on the recommendations of CLSI-GP47, which would help laboratory professionals to acquire a patient-risk based approach in AL development and CRR management.

SESSION 4

OP-10. New biomarkers for pregnancy

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Today, there are no widely accepted biomarker tests in clinical routine to predict the emergence of pregnancy complications in sufficient advance. Over tens of years, majority of efforts made by the scientific and clinical community have been targeted to prognostics of preeclampsia (PE), a multisystemic disorder affecting both the mother (extreme hypertension, proteinuria, impaired liver funciton etc) and the foetus (fetal growth restriction (FGR), preterm birth (PTB), perinatal mortality). Pathophysiology of PE is complicated and novel evidence indicates several subtypes of PE arising for different causes (1, 2). As the main cause behind PE is impaired placental development and function, profiling of placental differential gene/protein expression has been extensively utilized to compile the list of potential biomarkers for PE (3, 4). Currently, two commercial companies offer prognostic tests for PE based on analysis of two well-established maternal serum based biomarkers derived from placental malfunction, i.e. sFLT1 and PlGF (Roche Diagnostics, Thermo Scientific). However, these tests are expensive and do not have sufficient sensitivity and specificity for making long-term clinical decisions (5). One of the aims of the Happy Pregnancy (HP) project (http://www.happypregnancy.ut.ee/) has been to investigate and discover new maternal serum biomarkers for pregnancy complications, and to develop clinically applicable assays for them. The study is executed in collaboration of the University of Tartu, Tartu University Hospital and the Synlab Eesti Ltd. (former Quattromed HTI Laboratory Ltd.). The development of new biomarker tests builds on utilization of an excessive sample collection established within the HP project, including longitudinal blood and urine samples of >2000 pregnant women across normal and adverse pregnancy outcomes (PE, FGR, PTB, gestational diabetes). Based on the literature evidence and unpublished data, we have selected ~20 serum biomarkers reported to be significantly altered in PE. In the first stage, we are developing a method for simultaneous detection of multiple biomarkers in maternal serum and for evaluating their diagnostic potential to predict PE. The analytical characterisation of the pilot assay is currently ongoing. For the performance of two analytes, sFlt1 and PlGF, we were able to conduct a pilot study comparing the performance of our novel assay and the available commercial ELISA-based

SESSION 4

assays (Thermo Scientific B·R·A·H·M·S Kryptor). The two assays show very good correlation regarding measurements ($R^2 > 0.93$). Reference values, optimal timing of measurements, risk assesment and outcome prediction scores are in the analysis pipeline.

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OP-11. New trends in cardiovascular laboratory

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The first cardiac enzymes and the main aspects of lipoprotein metabolism were described in the 1950s and early 1960s. Now, there is universal definition of acute myocardial infarction, according to which use of high-sensitive troponins, in association with clinical evaluation and ECG for diagnosis is recommended. There are several troponin I/T-assays, which fulfil the criteria for high-sensitive troponin measurements. Today, a change in troponin levels (delta) is needed to show acute cardiomyocyte injury (0 and more than 3 hours). Also shorter protocols (1 hr and 2 hr) are studied and applied. With the application of high-sensitive Tns in two samples, there is less clinical need for other early markers (FABP, copeptin etc.), except in rule-out protocols. The CK-MB may be used for diagnosis of AMI in association with heart-operations (CABG or PCI). The POCT for Tns is demanding as the methods are analytically less sensitive than automated methods in core labs. In a regional health care system with common guidelines, the use of several markers and/or several cut-offs for rule-in or rule-out is also challenging. The use of natriuretic peptides is recommended as one of the tools for diagnosis of acute heart failure, if available. There are evidence-based rule-out and rule-in cut-off values for both N-ProBNP and BNP. New markers for heart failure are available and are evaluated for clinical use. The most promising new markers are GDF-15, galectin-3 and ST2. Based on recent studies they all are associated with chronic heart failure, complications and remodelling of the heart. Routine assays for the clinical laboratory will be available. Evaluation of cardiovascular risk is based on risk scores (including lipid profile). Apolipoproteins as risk markers have been studied for several decades. In some countries, apoB, instead of LDL, is recommended for clinical practice. There is also a current joint consensus statement by EAS and EFLM, which supports the use of non-fasting blood samples for routine testing, including the lipid profile. The corresponding applications are discussed.

SESSION 4

OP-12. Biomarkers in chronic kidney disease

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OBJECTIVES. Chronic kidney disease (CKD) is one of the leading public health problems. Cardiovascular diseases (CVD) account for 50% of all deaths in the CKD setting. Vascular calcification contributes significantly to higher mortality among this population. Disorders of Ca-P metabolism were regarded for many years as a major factor contributing to high cardiovascular risk in CKD patients. Proteinuria, especially together with eGFR, is a sensitive marker for progression of CKD but it has some limitations. Discovery of novel biomarkers changes the understanding of the pathogenesis of both progressions of CKD and CVD risk.

The aim of our study was to evaluate the levels of Human Fibroblast Growth Factor 23 (FGF23) and Matrix Gla Protein (MGP) in hemodialysis (HD) patients and assess its possible links with cardiovascular diseases and mortality.

METHODS. The study included 81 HD patients (44 men (54.3%) and 37 women (45.7%)) treated at the HD unit of Hospital of Lithuanian University of Health Sciences Kauno klinikos. Clinical data, FGF23, inactive form MGP and other laboratory data were analysed.

RESULTS. The mean age of patients was 60.9 ± 16.01 years (22–86) and the mean HD vintage was 39.26 ± 46.24 months (1–182). The duration of HD of 52 patients (64.2%) was more than a year, 17 patients (21%) were diabetic, 42 patients (51.9%) had cardiovascular diseases. During the follow-up period, 17 (20.9%) patients died.

Levels of MGP were significantly higher for patients who survived after 2 years of follow-up compared to patients who died (2.05 ± 1.42 vs. 1.38 ± 0.53 ng/ml, $p = 0.003$). Positive correlation was found between FGF23 and MGP ($r = 0.76$, $p < 0.001$).

CONCLUSION. According to our findings, hemodialysis patients with 2 -year survival had higher MGP values. These values and the unexpected findings of the positive correlation between FGF23 and MGP allowed to suggest that these novel biomarkers may play a role in the pathogenesis of cardiovascular diseases in hemodialysis patients. However, these findings require further research.

OP-13. New biomarkers of articular cartilage in patients with osteoarthritis

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BACKGROUND. Diagnosis of OA is based on clinical symptoms and radiological findings. How-ever, usually the latter describe already the advanced stage of the disease. Using biomarkers (BMs), it is expected to discover even the pre-radiographic disease, before the clinical illness. When investigating BMs one has to distinguish the two main radiogaphic features: osteophytes (OPH) and joint space narrowing (JSN).

METHODS. This report presents the findings of three biomarkers of knee OA:(1).Urinary type II collagen fragment (uC2C) reflecting cartilage degradation, (EIA) using a monoclonal AB that recognizes the carboxyl terminus of the 3/4 in the degraded alpha1(II) chain; (2). Cartilage Intermediate Layer Protein (sCILP)-2 in serum, assessed by an in-house competitive immunoassay, AnaMar AB, Sweden. The assay detects the C-terminal part of CILP-2 domain1 with polyclonal goat anti-CILP2 AB; (3). Cartilage oligimeric protein (sCOMP) reflecting general turnover of cartilage as well as othter joint tissues (commercial ELISA). The BMs were applied in the early phase of knee OA in a middle-aged cohort and relevant controls (36–62, mean age 50) both at baseline and after 3 years. Tibiofemoral (TF) and patellofemoral (PF) radiographs were graded for presence of OA.

RESULTS. Two BMs were able to distinguish OA patients with grade 2-3 and without structural changes. The COMP values were increased in patients with „progresssive" knee OA. After 3 years all three BMs showed stronger correlations with OPH compared with JSW. Unlike the other biomarkers, CILP-2 showed a decrease in patients with early grade of OA. In knee arthroscopy-detected cartilage lesions, a decrease of CILP2 was found already in grade 1 and an increase of uC2C, in grade 2.

CONCLUSIONS. *Longitudinal investigation of patients with definite forms of OA demonstrated that increased values of uC2C and decreased values of CILP2 could be associated with knee cartilage lesion, and progression of OA.*Cartilage neoepitopes offer new opportunities for in-depth investigation of the knee cartilage.

SESSION 5

OP-14. Patient-focused laboratory medicine. Lab4Patients Horizon 2020 project

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There is growing demand that patients be better informed and participate more actively in treatment related decisions. Initiatives to provide patients with access to their data, such as patient portals, reflect a patient-driven approach. The better informed patient is better equipped for participating in the medical decision process, and the terms *patient empowerment* and *shared decision making* are often used in this context. It has been shown that more active involvement of patients will lead to improved motivation to adhere to treatment, with a better health outcome. Many physicians are concerned that record access will bring about more work, with extra consultations and telephone calls as a result of patient misunderstanding. Studies suggest the opposite – that record access can reduce the demand for resource. Both the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and the Section of Laboratory Medicine of the UEMS (Union Européenne des Médecins Spécialistes) recognize the development of patient empowerment and the changing role of care providers and patients. To this end, the EFLM has established the Working Group Patient Focused Laboratory Medicine, that took the initiative of the current project.

The overall aim of the project is to design, develop and evaluate an interpretative knowledge and reporting system that will inform patients so that they can comprehend the significance of their laboratory test results, enabling them to better participate in the shared decision process with their physician. The system described in the Lab4Patients project represents a new integrated approach to process the data from other healthcare systems and communicate the results in a flexible way to different receiving systems and individual using several advanced types of communication.

The project is recently submitted; when accepted by the EC in Brussels it will help to reach a European standard in the translation of laboratory test information to information understandable for patients.

OP-15. Syndrome based test panels in hospital laboratory

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Different tools have been proposed to help clinical doctors in laboratory test requesting. At our hospital we developed syndrome based test panels in cooperation between clinical and laboratory doctors.

The panels were included in a computerised physician order entry (CPOE) system. For each panel, a pre-defined and optional set of tests was created. Requesting can be performed in one button click or tests can be added or removed in case of need.

Altogether 16 test panels were developed, 6 panels for the use at the hospital and 10 specifically for the Dept of Emergency Medicine (EM). In EM, test panels were designed for some of the most frequent acute clinical syndromes which included abdominal pain, shortness of breath, chest pain, monitoring myocardial markers, cerebral infarction with indication of thrombolysis, polytrauma, sepsis, chronic anaemia, urinary tract infection and joint pain.

At the hospital, test panels were implemented for cardiovascular risk assessment (atherosclerosis), venous thrombosis, antiphospholipid syndrome, contact case with patient's blood or body fluids, monitoring of clinical nutrition, and diagnosing malaria.

Syndrome based test panels help to save clinicians' time in a busy working environment and to avoid omitting of necessary tests in typical situations. Joint collaboration with clinical doctors enable better to introduce the test menu of the laboratory and to implement diagnostic guidelines.

SESSION 5

OP-16. Comparative whole genome hybridisation methods in molecular diagnostics

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OBJECTIVE. Comparative whole genome hybridisation (CGH) is currently the first-tier genetic diagnostic test for the detection of chromosomal imbalances in patients with congenital heart defects (CHD). The CHD as the most common congenital anomaly (approximately 1 in 100 newborns) has been reported to be frequently associated with pathogenic copy number variants (CNVs). Recently, CGH has been successfully applied to identify CNVs in postnatal and prenatal patients with CHD. This study aims to identify genomic imbalances in patients with syndromic and nonsyndromic CHD.

METHODS. Single nucleotide polymorphism CGH testing (HumanCytoSNP-12v2.1, Illumina) was carried out in 30 patients with syndromic and nonsyndromic CHD of unknown etiology, including 28 postnatal and 2 prenatal referrals. The testing is currently ongoing for other samples.

RESULTS. There were 8 clinical significant copy number variants identified in 7 patients. All these patients had additional phenotypic anomalies and/or intellectual disability. These CNVs included three *de novo* deletions, one deletion inherited from mother, one *de novo* duplication and three of yet unknown inheritance mode. Four of the pathogenic CNVs overlapped with known microdeletion / microduplication syndromes. No significant CNVs were detected in patients with isolated nonsyndromic CHD.

CONCLUSIONS. Our initial results contribute to the evidence that CGH has a high diagnostic yield and value for improved genetic counselling.

SESSION 6

OP-17. Management of sepsis in Estonian hospitals 2013-2014

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Sepsis is widely defined as a systemic inflammatory response to an infectious disease, caused by viruses, bacteria, fungi and protozoans. Sepsis, severe sepsis and septic shock are an important public health problem requiring urgent action and substantial human and material resources.

Prevalence rates of sepsis are estimated to be 30.7 million cases per year, in addition, 23.8 million severe sepsis cases. Nearly 6 million people die every year. The mortality rate of sepsis is 6.7%, it is higher in severe sepsis (20.7%) and the highest in septic shock (45.7%) (1). International guidelines for management of severe sepsis and septic shock (Surviving Sepsis Campaign) have been revised several times, most recently in 2012.

Estonia introduced national guidelines in 2009. These guidelines were approved by the Estonian Society of Internal Medicine, the Association of Surgeons of Estonia and the Estonian Society for Infectious Diseases. They were also approved by the Estonian Health Insurance Fund on 17.06.2010.

It is important to recognize sepsis because empirical antibiotic therapy should be started as soon as possible, within 1 hour. Although sampling should not delay timely administration of antimicrobial agents in patients with severe sepsis, obtaining appropriate cultures before administration of antimicrobials is essential to confirm infection and responsible pathogens and to allow de-escalation of antimicrobial therapy after receipt of the susceptibility profile. The Estonian Health Insurance Fund regularly examines the quality of services and the need for them, incl. approved guidelines. Management of sepsis at Estonian hospitals according to the national guidelines was the subject of audit in 2013–2014.

SESSION 6

OP-18. Mass-spectrometry for bloodstream pathogen identification in routine practice

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INTRODUCTION. In the case of suspicion of sepsis, rapid identification of bloodstream pathogen is essential to start adequate antibiotic therapy. In comparison with the conventional diagnostic procedure, application of mass spectrometry allows identification of a pathogen one day earlier.

OBJECTIVE. To describe the usage of MALDI-TOF Mass Spectrometry in November and December 2015 at the East Tallinn Central Hospital, and to compare the results of conventional culture and mass spectrometry methods for identification of bloodstream pathogens.

METHODS. Blood collected from patients with clinical suspicion of sepsis was first analysed using the blood culture (BC) system. From among positive BC bottles, one bottle per patient-episode (cases where BC was collected from the same patient at time points more than a week apart were regarded as separate patient-episodes) was processed using both the conventional culture and MALDI Sepsityper methods.

RESULTS. A total of 775 BC bottles (379 sets) from 216 patients were analysed with BC system, resulting in 90 positive bottles. At least one bottle was positive in 54 of 240 patient-episodes (23%); 53 bottles were processed further. According to the conventional culture, one pathogen was identified in 50, and two pathogens were identified in 3 cases. MALDI Sepsityper failed to identify any pathogens in 8 bottles of 53 processed (15%). In the remaining 45 cases, MALDI Sepsityper identified one pathogen. The pathogen was the same as identified by the conventional method in 43 bottles (81%); in 2 cases MALDI Sepsityper identified one of the two pathogens that had been demonstrated as being present by the conventional culture.

CONCLUSIONS. Mass spectrometry is a powerful method to speed up the ethiological diagnosis of sepsis in the routine practice of a microbiology laboratory. In our study, 43 of the 240 patient-episodes of clinically suspected sepsis (18% of all; 80% of all BC system positives), the pathogen was identified by MALDI Sepsityper one day earlier compared with the conventional culture.

OP-19. Diagnostic possibilities of fungal infections

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OBJECTIVES. Invasive fungal infections remain one of the most severe complications in immunocompromised patients. Early, sensitive and reliable diagnosis is mandatory. Clinical signs and symptoms of fungal infection are non-specific. Non-invasive diagnostic procedures are preferable because often patients are unable to undergo invasive diagnostic procedures. This study characterizes the current situation of diagnostic methods regarding the use and incidence of invasive candidiasis diagnosed in Estonia.

METHODS. The statistics of diagnosed cases of invasive candidiasis, invasive aspergillosis, mucormycosis and cryptoccosis in different patient groups, based on the data of the Estonian Health Insurance Fund database, is presented for a 5-year period (2010–2015). Antifungal susceptibility data about candidemia were collected from the records of Tartu University Hospital. A questionnaire on the diagnostic methods used at microbiology laboratories was sent in 2000 and 2015, development of diagnostic methods is compared.

RESULTS. The blood culture still remains the main diagnostic tool for invasive candidiasis. The practice of performing blood cultures has improved over the 15 year period. Screening for circulating antigens like galactomannan or other polysaccharides has been proved to be beneficial in preventing the severe stage of fungal infection. Centralized laboratory service has allowed to use more diagnostic tests and a complicated diagnostic approach in case of rare infections.

CONCLUSIONS. The results highlight the need to apply novel immunological and molecular methods at least at regional hospitals.

SESSION 7

OP-20. Standardization of laboratory tests in Lithuania

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OBJECTIVES. The aim of the presentation was to describe the current situation in the standardization of laboratory tests at Lithuanian laboratories and health care institutions. The need for standardization was recognized by several e-Health project groups whose aim was setting-up of common platforms for ordering laboratory tests by healthcare professionals of different institutions and for pooling lab tests results so that they would be available for use irrespective of where a test was performed.

METHODS. A group of laboratory professionals from all subspecialties of laboratory medicine has been established by the Board of Lithuanian Society of Laboratory Medicine. The LOINC nomenclature was selected as a general background for compiling a list of tests and measurement procedures most commonly used by local labs.

RESULTS. It was identified that the LOINC nomenclature was too complex for many small labs and primary health care providers; also the national laboratory system has different traditions for grouping, ordering tests and reporting results. Laboratory tests were divided into 8 groups according to the main subspecialties of laboratory medicine and a unique coding system was established. Only the measurement procedures recommended by standards and by internationally recognized organizations (IFCC, CLSI, etc.) were included with the aim to prove the situation where out-of-date testing principles with limited specificity or sensitivity are still widely used. It was later recognized that very strict methodological limitations can be very inconvenient for labs and can lead to an abrupt increase of costs. Therefore, in the case of some test methods, which are not up-to-date, a reasonable transition period with an annual revision of the nomenclature was foreseen.

CONCLUSIONS. The developed nomenclature will improve laboratory services for patients and health care providers through standardization of laboratory tests and methods as well as will simplify communication between the e-Health subsystems.

OP-21. LIS and eHealth in Finland

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Along with the evolution of eHealth also the Laboratory Information Systems is in the middle of a significant change. General eHealth drivers: cost of care, ease of use and faster decision-making set the pace also for LIS development. The borders between digital health services are disappearing, which changes the health care processes supported by seamless and timely integration of information from various sources – including laboratory diagnostics. Furthermore, the processes *inside* the laboratory diagnostics are getting closely integrated and all this is efficiently supported by integrated LIS implementation.

Health care IT in Finland is facing great changes: huge public EHR projects are being planned. Interoperability, integration and transfer of information play the key role in embracing new customer-oriented eHealth solutions. Also the interest of the government in eHealth is more pronounced. An example of this is the central storage of health data (Kanta) integrating data from public and private EHRs, to be used by consumers and professionals.

The basic function of the LIS, from the point of view of the clinician, has been rather simple: *getting orders and delivering results*. Both of these ends have started to become much richer. We can add intelligence to both clinical and lab resource management, provide more information beyond plain results and support medical decision-making. All this is becoming available for the consumers as well. Self-service appointments and access to own lab results are examples of this development. This trend of professional and consumer experiences is moving closer to each other, *consumerization*, has truly reached health care as well: professional services are getting commoditized, consumers are getting self-service access to professional information and consumer-style approaches are re-shaping professional usage. Services are mobile, they are web based and developing fast. Costs are indeed decreasing, usage is easier and decision making faster.

SESSION 8

OP-22. Interpretation of syphilis serodiagnostic tests: looking from the clinician`s side

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OBJECTIVES. The subject of this presentation is the knowledge of currently used serologic tests for syphilis, and of an ideal serologic test for syphilis, and comparison of current syphilis serology tests.

METHODS. We describe limitations in interpretation of syphilis serology results and the consequences of suboptimal values of serology tests; also how false results of non-treponemal and treponemal tests come into being and why a two-test diagnostic approach for the serodiagnosis of syphilis is necessary.

RESULTS. The enzyme linked immunospot assay for treponema pallidum specific antibody secreting cells and the line immuno assay represent new diagnostic possibilities of syphilis serology.

CONCLUSIONS. It is evident that one has to take account of limitations in the interpretation of syphilis serology. These limitations comprise the suboptimal sensitivity and specificity of the complex immune response of the host to Treponema pallidum, the passive transfer of anti-treponemal IgG antibodies through the blood-brain barrier and the placenta and the abnormalities of serological response to Treponema pallidum in immune deficiency patients. Further developments, molecular biology techniques, standardisation and automation, will contribute to more simple and more precise interpretation of syphilis serology.

OP-23. Application of syphilis laboratory diagnostics in Latvia

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OBJECTIVES. Serological tests are the mainstay in the diagnosis and follow up of syphilis. Detection of *Treponema pallidum* by the dark-field microscope or PCR is suitable for lesion exudates or tissue. Guidelines contain recommendations for the laboratory diagnosis of syphilis. For syphilis serological diagnostics, tests are used according to the 2014 European Guidelines in the Management of Syphilis. The aim of this study was to follow up guidelines of syphilis serological diagnostics in Latvia.

METHODS. From 2013 to 2014 there were tested 9650 samples at the laboratory of Latvian Center of Infectious diseases. We used syphilis serological tests such ELISA (Abbott diagnostics, USA; Mikrogen diagnostic, Germany), TPHA (newMarket Laboratories, United Kingdom), IFAabs (bioMerieux, France), IB (Mikrogen diagnostic, Germany) and RPR (Lab21 Healthcare, United Kingdom).

RESULTS. For syphilis screening, we used ELISA test. Altogether 9650 samples were tested by ELISA, among them the number of positive samples was 1032. ELISA positive samples were re-tested using TPHA. A total of 979 samples were TPHA positive, coincidence between the ELISA and TPHA was 94.8%. Fifty- three TPHA negative and ELISA positive samples were tested using IFA or IB in case clinicians ordered testing. We performed ELISA IgM, in case there were suspicious cases of early syphilis. Dermatovenerologists ordered both RPR and ELISA in cases of suspected of very early syphilis.

CONCLUSIONS. Laboratory serological diagnostics of syphilis is based on the 2014 European Guidelines on the Management of Syphilis. Treponemal ELISA has become the test of the first choice for syphilis screening. Laboratories should collaborate with dermatovenerologists in serological testing of syphilis.

SESSION 8

OP-24. Sexually transmitted infections in Lithuania

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In Lithuania there are three notifiable bacterial sexually transmitted infections (syphilis, gonorrhoeae and chlamydial infection) and one viral (HIV). The STIs are diagnosed and managed following the recommendations of IUSTI guidelines and reported if a person meets clinical, laboratory and epidemiological criteria. Laboratory criteria are crucial for notifiable STIs. The main routine laboratory tests such as microscopy of genital smears, serology for syphilis (RPR, TPHA), serology for HIV are available in the laboratories of bigger hospitals or even primary health centers. Laboratory tests for complicated STIs (e.g. VDRL-CSF for neurosyphilis, TP IgM antibodies for congenital syphilis, PCR for congenital herpes infection) as well as antibiotic resistance control are concentrated at the laboratories of the university hospitals and National Public Health Surveillance Laboratory (NVSPL). Different molecular tests for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomona vaginalis*, *Mycoplasma genitalium*, ureoplasmas are introduced in university hospitals, NVSPL and private laboratories. Nucleic acid amplification tests for STIs are not covered by health insurance and the patients have to pay for them.

The incidence rates over the last 3 years (2012–2014) for syphilis ranged from 7.6 to 8.8 cases/100000 population, for gonococcal infection, from 7.3 to 5.6 cases, and for chlamydial infection, from 8.9 to 15.3 cases. In recent years several epidemiological projects on STIs in Kaunas have shown that young people actively seek for timely STIs testing and treatment. In 2013 at the Hospital of Lithuanian University of Health Sciences, a cross sectional study was designed. The participants completed a standardized questionnaire. First voided urine (FUV) samples were analysed for the presence of 7 urogenital pathogens: *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonasvaginalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Ureaplasma parvum* by using the multiplex real-time PCR assay "Anyplex TM II STI-7 Detection" (Seegene, Inc., Seoul, Korea). The results showed that the frequency of STIs among 963 patients was: 6.2% for chlamydial and 0.4% for gonococcal infections, 2.7% for *Mycoplasma genitalium* and 0.5% for *Trichomona vaginalis* infections. In the age group 20–30 years *C. trachomatis* was detected in 10.2% of women, in the younger than 20 years age group, in 6.25%, and in the age group of 30–40 years, in 4.69% of women. *Chlamydia trachomatis* was more common among women who had more than two sexual partners in the last half-year. Most of the infected persons did not want to give information about their sexual relationship with other partners.

SESSION 9

OP-25. Lymphocyte subsets and serological markers in melanoma patients treated with ECHO7 virus

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OBJECTIVE. To evaluate changes in immunbiological markers during one year of virotherapy and recurrence or metastases in patients who have undergone radical melanoma excision and have received treatment with ECHO-7 virus *Rigvir*.

METHODS. 70 patients with skin melanoma stage IB and II who had undergone radical melanoma excision at the REUH Oncology Centre of Latvia in 2012 were analyzed. The CD3+, CD3+4+, CD3+8+, CD16+56+, CD19+, CD38+, CD95+ cells, S100 and LDH were evaluated in peripheral blood (PB) and T subsets were evaluated in tumour tissue. Ly subsets were determined before starting the therapy and at 1, 3, 6, 9 and 12 months during the therapy. Statistical analysis of the data was carried out using the SPSS statistical software (V.20).

RESULTS. The incidence rate of metastases or disease recurrence in patients who received Rigvir was 21.4%, while in the control group it was 30.1% during the three years of follow-up. Sensitivity of S100 and LDH increased with the progression of the disease in visceral organs. After 1 month of Rigvir therapy an increase of CD16+, CD19+ and CD38+ and CD95+ cells was observed most often (46.2% and 61.5% of cases, respectively). After 3months of treatment 52.9% of the patients showed increased CD19+ and CD38+ cell count in PB. 6 months after the initiation of treatment CD4+Ly count was increased in 66.7% of cases, CD8+Ly count in 73.3%, CD19+Ly count in 68.8%, and CD19+Ly count in 75% of the cases. The cell count that expresses activation markers CD38+ and CD95+ had increased in 75% and 68.8% of the patients, respectively. After 9 months of treatment, CD16+ and CD19+ cell count had increased in 47.7% and 58.8% of the cases, but after the first year of treatment CD8+ and CD19+ cell count had increased in 58.3% of all cases.

CONCLUSIONS. The majority of patients who received *Rigvir* therapy showed an increase in the number and activity of T Ly subpopulations and an increase in B Ly and NK 6 months after the initiation of treatment, indicating marked activation of cellular immunity. The patients who were treated with ECHO 7 virus *Rigvir* were found to have less often recurrence or metastases, compared to patients who were followed up by observation.

OP-26. Colorectal cancer, new immunotherapy

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Colorectal cancer (CRC) is the fourth most common cause of cancer death after lung, liver and stomach cancer. If CRC metastasises, and the chances of radical surgery are eliminated, the prognosis is poor. In 2004 the company DanDrit Biotech* launched a study (Colorectal Cancer Trial) to evaluate the clinical and immunological effects of treating patients with disseminated CRC using a dendritic cell based cancer vaccine (MelCancerVac).

MelCancerVac® (MCV) was developed as a personalized medicinal product by DanDrit Biotech, for treatment of advanced CRC. According to European regulation 1394/2007 (article 17), MCV is expected to belong to the category of cell-based medicinal products and could be considered an Advanced Therapy Medicinal product (ATMP) candidate. MCV is produced for a Phase IIB/III clinical trial and for Patient Name Use program by Cellin Technologies LLC** (Tallinn, Estonia).

MCV is a polyvalent vaccine targeting shared tumour-specific antigens. MCV uses patient's own dendritic cells loaded with tumour antigens from the lysate of a specifically selected melanoma cell line. A melanoma cell lysate (MCL) is used as a source of tumour antigens. The melanoma cell line used to generate the MCL was selected because it expresses a well characterized family of shared tumour specific antigens, the MAGE-A genes. The MAGE-A genes have been extensively studied and are among the best-characterized tumour antigens. This family of genes is expressed in many different types of cancer, making them an attractive target for immune therapy.

*) DanDrit Biotech USA, Inc. is a biotech company, founded in 2001, focusing on clinical development of dendritic cells vaccine for treatment of colorectal cancer, which is ready for phase III clinical trials.
**) Cellin Technologies LLC is a cell therapy products contract manufacturing company founded in 2008. They hold a manufacturer's authorisation for preparing sterile products and an activity licence for handling cells, tissues and organs, issued by the Estonian State Agency of Medicines (SAM).

SESSION 10

OP-27. Emergence and spread of antibiotic resistance – not only a medical problem

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Antibiotics are not only used in human medicine. In fact, more than half of the amount of antibiotics produced is used in animal husbandry and veterinary practice. Thereby, antibiotic resistance can emerge both in medicine and animal husbandry. Resistant bacteria and resistance genes can be transferred between different settings. The surrounding environment often provides here an important link. Our aim was to compare the bacterial strains isolated from these settings and thereby to identify potential routes for transfer of resistance. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Enterococcus strains were collected and characterized by full genome sequencing. In many cases very similar bacterial strains were isolated from humans, animals and the environment suggesting efficient transfer routes.

The data will be discussed in the context of observations from other countries and the benefits of whole genome sequencing of bacterial strains will be underlined.

OP-28. Epidemiology of AB resistant gram-negative bacteria in the Baltic Sea region

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INTRODUCTION. The recent EARS-Net report shows that there is a general Europe-wide increase of antimicrobial resistance in the gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*. Production of extended-spectrum beta-lactamases (ESBLs) is one of the most common resistance mechanisms of gram-negative bacteria. Within the Baltic ESBL Epidemiology project (BEEp) ESBL positive *E. coli* and *K. pneumoniae* clinically relevant isolates were collected in Estonia, Latvia, Lithuania, Norway and the Saint Petersburg region in Russia.

OBJECTIVE. To describe the prevalence of ESBL producing isolates and to determine the molecular epidemiology of ESBL positive *E. coli* and *K. pneumoniae* in the Baltic Sea region. Investigations of spreading of some successful clones between institutions and countries were applied.

METHODS. Clinically relevant isolates collected during January–May 2012 in the Baltic countries, Norway and Saint Petersburg were analysed. Molecular techniques (PCR, RT PCR, whole genome sequencing), MALDI-ToF typing and statistical methods were applied to the data.

RESULTS. A total of more than 13000 isolets were screened and 1047 were confirmed as ESBL positive. The $ESBL_A$ phenotype among *E. coli* was the most prevalent (n = 362/471) with 77% and was followed by strains with both phenotypes ($ESBL_A$ and $ESBL_M$) (n = 69/471) at 15% and $ESBL_M$ (n = 40/471) at 8%. The $ESBL_{CARBA}$ phenotype was confirmed at 2.6% of *K. pneumoniae* isolates (n = 15/576) with carrying bla_{NDM} gene. There was found high prevalence of CTX-M-15 followed by CTX-M-14 in *E. coli* and CTX-M-15 followed by CTX-M-1 in *K. pneumoniae* isolates.

CONCLUSION. This study demonstrated the possible affection of the Baltic countries, Norway and the St. Petersburg area by the global ESBLs pandemic. Our study showed the high prevalence of CTX-M group genes in different combinations within group and with other genes in clinical *E. coli* and *K. pneumoniae* strains from Estonia, Latvia, Lithuania, Norway and Saint Petersburg.

SESSION 10

OP-29. Management of antibiotic resistance at the largest university hospital of Lithuania

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The increasing rates of multidrug resistant microorganisms (MDRO) make options for treating patients with these infections extremely limited. Increased length of stay, costs, and mortality have also been associated with MDRO. The recommended control interventions to reduce the rates of MDRO include administrative support, rational use of antimicrobials, surveillance (routine and enhanced), Standard and Contact Precautions, environmental measures, education and decolonization.

ADMINISTRATIVE SUPPORT. Documents and committees for regulating control interventions in hospitals.

EDUCATION. Educational campaigns to enhance adherence to hand hygiene practices in conjunction with other control measures have been associated temporally with decreases in multidrug resistant microorganisms` transmission in various healthcare settings,

Rational use of antimicrobial agents. Strategies for influencing antimicrobial prescribing patterns within healthcare facilities include education; formulary restriction; prior-approval programmes, including pre-approved indications; automatic stop orders; academic interventions to counteract pharmaceutical influences on prescribing patterns, antimicrobial cycling, computer-assisted management programmes and active efforts to remove redundant antimicrobial combinations.

MDRO SURVEILLANCE. The simplest form of MDRO surveillance is monitoring of clinical microbiology isolates resulting from tests ordered as part of routine clinical care, MDRO infection rates, molecular typing of MDRO isolates, surveillance for MDROs by detecting asymptomatic colonization.

INFECTION CONTROL PRECAUTIONS. Contact precaution and cohorting and other MDRO control strategies.

ENVIRONMENTAL MEASURES. Need for environmental cultures. Methods for evaluation the quality of environmental cleaning practices.

Decolonization.

OP-30. PCR-based identification and serotyping of streptococcus pneumoniae strains and antibacterial sensitivity assessment in Latvia

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INTRODUCTION AND AIM. Streptococcus pneumoniae, or pneumococcus, is a frequent cause of otitis media, respiratory infections and community acquired pneumonia. The aim of the study was to determine and compare serotypes of S. pneumoniae using Neufeld reaction and the capsular sequence typing method (CST) in 2015 and to determine antibacterial sensitivity in 2011–2015.

MATERIALS AND METHODS. The CST is based on a part of the sequence of the wzh gene of the capsular locus. In 2015 we performed CST for 17 S. pneumoniae strains with already known serotypes and in 2011–2015 we made 108 antibacterial sensitivity assessments.

RESULTS. Using the CST method we obtained capsular serotypes with the corresponding S. pneumoniae serotype: 3 capsular serotypes 04-03 (4), 2 07F-01 (7F), 2 09N-01 (9N), 2 19A-01 (19A), 1: 03-05 (3), 10A-01(10A), 15F-01(15F), 19F(19F), 22F-01 (22F), 23A-01(23A), 35B-01(35B), 35F-01(35F). Analysis of the data showed that 19 of 108 S. pneumoniae strains had antimicrobial resistance: 4 strains (3.7%) to penicillin, all being serotype 14; 15 strains (13.9%) to sulfamethoxazole/trimethoprim, of which 14 were serotype (6 cases), 23F (2 cases) and 6B, 7F, 9N, 10A, 11, 15B, 19F (one case of each serotype).

CONCLUSION. The CST and pool antisera performing Neufeld reactions showed the same corresponding serotype. Development of PCR-based serotyping systems has the potential to overcome some of the difficulties associated with serological testing. Resistance to penicilin in Latvia is not as high (3.7%) as in other countries and belongs to serotype 14 only, However, resistance to sulfamethoxazole/trimethoprim accounts for 13.9% of all the strains and belongs mostly to serotypes 14 and 23F.

W-1. Workshop on the microscopy diagnostics of urogenital tract diseases

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OBJECTIVES. The workshop is conducted with the purpose of education, upgrading qualification and maintaining professional skills of physicians, contributing to their systemic view on the microscopy diagnostics of inflammatory and non-inflammatory diseases of the urogenital tract. The target audience of the workshop includes dermato-venereologists, obstetricians and gynecologists, urologists, infectionists, family physicians and other specialists using microscopy at their offices, as well as microbiologists and specialists in laboratory diagnostics.

METHODS. Practical use of microscopy methods for the evaluation of the vaginal microbiocenosis, diagnostics of bacterial vaginosis, urogenital candidosis, trichomoniasis, as well as the assessment of inflammatory reactions of the vaginal and cervical epithelium, enables to make a diagnosis already during the patient's first visit and to choose further examinations needed and adequate treatment. At the workshop, the theoretical basis of light microscopy is discussed, as well as the structure of the light microscope, techniques of correct sampling for microscopy of wet mount and stained preparations, methods of staining preparations, microscopy in transmitted light, evaluation of microscopy data, and interpretation and reporting of microscopy results.

RESULTS. Improvement in theoretical knowledge and gaining practical skills in direct microscopy for the evaluation of urogenital microflora and diagnostics of some sexually transmitted infections, as well as bacterial vaginosis, urogenital candidiasis and such inflammatory diseases as urethritis, cervicitis.

CONCLUSIONS. The workshop is an important part of the education of physicians who plan to use direct microscopy in their practical work.

W-2. Workshop on method verification

Anders Kallner – Department of Clinical Chemistry, Karolinska University Hospital, Stockholm, Sweden

Thinking about method verification in your lab or how to establish and monitor quality in the laboratory? This workshop will deal with simple statistical principles and techniques which are necessary to understand and apply in laboratory medicine. There will be a short introduction to the use of spreadsheet programs on computers. After this brief refreshment of metrological basic knowledge and understanding, we will outline the principles and methods for estimating and monitoring quality, as well as the limitations of the results of measurements, with a particular focus on validation of measurement procedures.

CP-3. New era in point-of-care glucose testing in the hospital: regulatory changes and clinical significance

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Point-of-care (POC) glucose meters are daily used in hospitals around the world. These meters, often designed for consumer use and introduced in hospitals at a later stage, pose significant safety risks to patients due to interferences that can cause meters to over-report glucose, resulting in over-dosing of insulin and an increase of hypoglycemic events. In 2007 the first glucose meter designed for hospitalized patients, which corrected for all interferences such as hematocrit, electrochemical and non-glucose sugar interferences, was introduced, providing a reliable resource to address this unmet medical need.

This presentation will focus on:

- Discussing the significance of point-of-care (POC) glucose testing for hospitalized patients
- Defining the problems caused by inaccuracy of glucose meters
- Presenting clinical cases showing the importance of accurate POC glucose testing in hospital
- Discussing the new regulatory requirements for bedside glucose measurement systems (BGMS) in hospitals
- Presenting clinical evidences supporting the role of accurate POC glucose testing in proper glycemic management

CP-5. The use of allergen component IgE testing

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Allergens are antigens, usually proteins, which are able to bind to specific IgE producing consequently allergic reactions. Each living organism is a potential source of allergens and each source of allergens contains numerous proteins that are called allergen components. Allergen components can be grouped into different protein families based on their structural similarity. Some allergen components are extremely specific to the allergen source and some are widely cross-reactive between different allergen sources. Allergen components differ also regarding their stability. Some proteins withstand processing reasonably well, but others denature readily, for example, by heating. The specificity and stability of allergen components are strongly connected to symptom probability and severity.

Molecular allergology, i.e. determination of allergen components, provides more precise tools for clinical risk assessment. For example, as an allergen source, peanuts contain both specific stable allergen components and cross-reactive unstable components. Persons who are sensitized to specific components are at much higher risk for severe allergic reactions compared to those who are only sensitized to non-specific cross-reactive components.

Symptoms elicited by cross-reacting antibodies can lead to unnecessary avoidance of particular food products. By distinguishing unspecific sensitization from genuine sensitization it is possible to provide patients with adequate advice on avoidance and symptom management. In cases of cross-reactive sensitization it is advisable to identify the primary sensitizer by further testing. The exact identification of genuine sensitization to the relevant allergen source is also essential when specific immunotherapy is considered. Component resolved IgE testing will considerably considerably improve diagnostic accuracy and patient management.

CP-6. The next generation in rapid, point of care testing

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Sofia, the next generation in diagnostic testing, takes rapid diagnostic testing to a new level by integrating proven lateral-flow technology, proprietary advanced fluorescence chemistry and assay development techniques - all of these incorporated into a small bench top analyzer that can be used near patient and in laboratory settings.

During this session one can see that Sofia tests are easy to use and can be adapted to the needs of any healthcare setting. From the small physician office laboratory to larger hospital labs or emergency departments, this discussion will highlight the high performance, objectivity, quality control, fail-safe measures, LIS capabilities and expanding test menu in respiratory diseases, women's health, and other areas of testing which make Sofia analyzer a perfect solution for the laboratory staff and physicians whose aim is to improve their rapid, point-of-care testing algorithm.

CP-7. How to improve clinical diagnostics in hospital emergency departments

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The concept of triage is the most clear-cut difference between diagnostics in emergency departments (ED) and in other fields of medicine. Decisions are here made on small, standardised sets of clinical and biochemical parameters and not on extensive investigations.

Continuous development of panels of appropriate tests for specific acute conditions is a fruitful way to improve diagnostics. The preanalytical phase of laboratory diagnostics must also be high on the agenda. The post-analytics of IT and interpretation should be discussed.

Turn-around time is important but should not be over-emphasized. While some condition requires results within minutes, the majority do not. However, it is essential that the turn-around times are consistent and predictable.

Improving the quality of an ED is not a single person's job. The key persons of the department must be identified. The different priorities of nurses and doctors should be raised. The laboratory and the ED will then work in multidisciplinary groups where lab doctors focus on emergency physicians, and lab scientist, on emergency nurses. Laboratory leadership should establish good relations with the leaders in ED. The staff turnover at EDs is among the highest in healthcare and presents additional challenge when starting collaboration.

In all discussions, the laboratory staff should be prepared to listen to what clinicians really want. And also be prepared to accept solutions that are not normally on the agenda, or even accepted, at a scientific laboratory. The use of venous blood gases, by means of POCT, in the triage at EDs is one of several examples that I will discuss in my presentation.

CP-8. Procalcitonin and antibiotic management

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The role of PCT in assisting antibiotic therapy has been studied extensively, with contradictory results. However, to understand the values and limitations of PCT in antibiotic management, it is inevitable to understand the immunological background of critical illness. The PCT distinguishes bacterial infections from SIRS; however, individual interpretation of a single elevated PCT could sometimes be very difficult. A study of Clech showed that medical patients with infection (PAMP) should have lower PCT values as compared to surgical patients with infection (DAMP+PAMP) in whom PCT elevation was found to be 5-10 times higher. The kinetics of PCT is always over a single PCT value in case of antibiotic commencement. In a recent pilot study we measured PCT before initiating antibiotics and further 8 hourly (t8, t16, t24) after commencing empirical antibiotic therapy, and found a significant difference in the kinetics between patients receiving appropriate treatment and those receiving inappropriate antibiotic therapy. A PCT elevation of ≥55% within the first 16 hours (i.e. from t0-t16, AUC 0.78) was associated with reduced hospital mortality (35% for the appropriate therapy vs. 65% for the inappropriate therapy group). The oversized adaptive immune response can induce the immunoparalysis condition. According to Rau, patients with secondary peritonitis had re-increased PCT levels indicating infection, but the peak values were found to be significantly decreased. This result was confirmed by Charles who found that in medical patients PCT was higher during primary infection, but it was several times lower during a second infectious insult despite the same clinical gravity. These data indicate that in the immunoparalysis condition lower levels of PCT should be taken just as seriously as high levels in the early course of the disease. In a landmark study by Christ-Crain, antibiotic exposure was reduced safely by 50% in patients admitted to emergency wards with acute respiratory complaints, when antibiotic therapy was guided by admission PCT levels. Two subsequent multicenter trials also found a safe decrease in antibiotic exposure in patients treated for infections in ICU. In conclusion, PCT kinetics in a multimodal approach helps us to start, continue, or change antibiotics and, most importantly, to stop them.

PP-1. Copy number variation at chromosome 16p13.11 in Estonian patients

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OBJECTIVES. There are many copy number variations (CNVs) that are associated with susceptibility for neurodevelopmental disorders. One such novel CNV is deletion and duplication at chromosome 16p13.11 whose clinical significance is becoming more firmly ascertained.

The CNVs at 16p13.11 show a broad range of phenotypic manifestations and incomplete penetrance. They are significantly enriched in individuals affected by developmental delay/intellectual disability, autism, epilepsy, dysmorphic features and congenital anomalies. Pathogenic 16p13.11 CNVs vary in size, but harbor the critical region called interval II (chr16: 15.48-16.32 Mb, GRCh37/hg19).

METHODS. We investigated the burden of CNVs at 16p13.11 (HumanCytoSNP-12 v2-1 BeadChips; Illumina Inc.) in a sample of 3,212 individuals with a range of neurodevelopmental conditions clinically referred for chromosomal microarray analysis. The ases were compared with 14.747 controls from the Estonian Genome Center. We identified 16 patients with CNV within the 16p13.11 region accounting for ~ 0.5% of the analysed patients versus ~ 0.15% of the Estonian general population. Eight cases were with deletion and eight were with duplication in this region, including one prenatally diagnosed case.

RESULTS. We found that patients with CNV in 16p13.11 present with varied clinical features as previously described. These features are incompletely penetrant. All deletions and duplications identified encompass the critical region of the CNV. The sizes of the rearrangements vary between 0.3–2.7 Mb.

CONCLUSION. Our findings confirm that genomic abnormalities at chromosome 16p13.11 predispose to a range of neurocognitive and developmental disorders individuals who carry them.

PP-2. Frequency of red blood cell alloantibodies among patients at the Hospital of Lithuanian University of Health Sciences Kauno Klinikos

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OBJECTIVES. Alloantibodies occur as immune response to foreign antigens. Identification of alloantibodies to red blood cells (RBC) in patients is important as it can help prevent blood transfusion complications. Our study was carried out in order to determine the frequencies of RBC alloantibodies by routine antibody screening and their identification in men and women who underwent transfusion therapy.

METHODS. RBC alloantibody screening and identification by gel column agglutination method (BioRad, Germany) was carried out from January 2010 to January 2014 in 2700 patients: 1456 male and 1244 female. The mean age of the patients was 48 years (range 18–78 years).

RESULTS. Screening for alloantibodies to RBC antigens was positive in 11.4% (n = 308) patients. Positive results were more frequent in women than in men (46.1% and 53.9%, respectively; p < 0.001). The frequency of alloantibodies to different blood group antigen systems is shown in the table.

Table. Frequency of antibodies in relation to blood group antigen systems according to patients` gender

Blood group antigen system	Frequency of alloantibodies		χ^2	p
	Gender			
	Men n = 1456 n (%)	Women n = 1244 n (%)		
Rh	72 (4.9)	167 (13.4)	59.78	**< 0.001**
Kell	33 (2.3)	88 (7.1)	36.22	**< 0.001**
Duffy	8 (0.5)	5 (0.4)	0.31	0.581
Kidd	7 (0.5)	3 (0.2)	–*	0.358
Lewis	20 (1.4)	19 (1.5)	0.11	0.739
P	1 (0.1)	1 (0.1)	–*	0.999
MNS	7 (0.5)	11 (0.9)	1.65	0.199
Lutheran	36 (2.5)	64 (5.1)	13.43	**< 0.001**
Xg	2 (0.1)	1 (0.1)	–*	0.999

* Fisher exact test was employed for small sample size.

CONCLUSIONS. RBC alloantibodies were more frequent in women than in men. The most frequent antibodies were against Rh, Kell, Lutheran blood group antigen systems. The frequency of Rh, Kell and Lutheran alloantibodies in women was higher compared to men.

PP-3. Gene polymorphism investigation of methylenetetrahydrofolate reductase (MTHFR) enzyme in chronic lymphocytic leukemia (CLL)

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Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme regulating intracellular folate metabolism which plays an important role in carcinogenesis through DNA methylation. In this study, MTHFR gene A1298C (rs1801131) and C677T (rs1801133) polymorphisms were determined in a case–control study of 91 patients with chronic lymphocytic leukemia (CLL) and 101 healthy control subjects using a real-time polymerase chain reaction (RT-PCR).

The allele and genotype frequencies of MTHFR gene A1298C polymorphism were compared between the control and the case groups. In statistical analysis, the A allele and the AA genotype were significantly found to be a protective variant. There were significant associations between the control and the case groups in C allele and CC genotype of A1298C polymorphism ($p = 0.04$ and $p = 0.03$). The 1298CC genotype increased 2.58-fold the risk of CLL.

The allele and genotype frequencies of MTHFR gene C677T polymorphism were compared between the control and the case groups. There was no association between the control and the case groups for the allele frequencies of C677T polymorphism. However, in statistical analysis, 677CT and 677CC genotypes significantly increased the risk of CLL ($p = 0.04$, OR = 4.21 (1.10–16.09) and $p = 0.05$, OR = 3.77 (1.00–14.17), respectively).

When the haplotype analysis was performed, there were significant differences between the case and control groups in terms of haplotype blocks. The AC haplotype block (A1298 ve C677) showed a protective effect against the risk of CLL ($p = 0.034$).

Our results demonstrate that MTHFR gene 1298C and C677 alleles have a major effect on the risk of CLL.

PP-4. Biochemistry-based analysis of prenatal diagnosis assessing the risk of congenital fetal development disorders

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INTRODUCTION. Biochemical analysis of serum is an important non-invasive test for detecting patients with an increased risk of congenital malformations. Modern serum analysis helps to identify pregnant women who are at risk of neural tube defects, 21 and 18 chromosome trisomy (Down and Edwards syndromes).

OBJECTIVE. To evaluate the results of changes in biochemical blood markers in prenatal diagnostics.

METHODS. Analysis of 1118 pregnant women's (mean age 33 ± 5 years), tested at the LUHS medicine laboratory in 2014–2015, the results of biochemical blood tests intended for prenatal screening. First trimester markers (n = 927) (double test): plasma protein A (PAPP-A) and free β-chorionic gonadotropin (β-hCG) analysed in weeks 11–13 and/or second trimester markers (n = 384) (triple test): alpha-fetoprotein (AFP), chorionic gonadotropin (hCG), unconjugated estriol (uE3) analysed in pregnancy weeks 14–20. Investigations were carried out with the Delfin 6000 Xpress analyser using the immunometric method, Wallace Oy DELFIA Xpress (Finland) reagent kits. Statistical analysis of the data was performed with the IBM SPSS 20 program. The patients were divided into 4 age groups (under 30, 30–34, 35–37 and over 37 years.). Differences between the groups were analysed by ANOVA statistical method. Data were statistically significant at $p < 0.05$.

RESULTS. In the first trimester pregnant women aged up to 30 years were found to have lower β-hCG, and in the second trimester, higher concentration of AFP, compared with the age group of 30–34 years, and increased uE3 concentrations compared with all older age groups. Higher concentrations of β-hCG and lower concentrations of uE3 were found in the blood of primigravida aged 30–34 years.

CONCLUSIONS. Pregnant women aged 30–34 years showed major biochemical abnormalities, especially in the first pregnancy, which indicates the potential risk of fetal pathology, and in pregnant women aged up to 30 years the risk of congenital abnormalities in fetal development on the basis of biochemical blood markers was the lowest.

PP-5. MMP-9 as a new marker to predict coronary heart disease in native Lithuanians

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OBJECTIVES. Coronary heart disease (CHD) is the leading cause of death in the developed world. Traditional factors of this disease are known, but there is search for new markers, and they are under investigation. Matrix metalloproteinase 9 (MMP-9) is one of new potential biomarkers.

METHODS. We have investigated 80 healthy adults and 77 with self-reported CHD from different regions of Lithuania. They belong to the second generation living in the same region of Lithuania; their parents are from the same ethno-linguistic region. Diagnosis was confirmed on the basis of medical records.

Serum samples were analysed for total cholesterol (T-C), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), ApoAI, ApoB, hs-CRP, IL-1 beta and MMP-9. For statistical analysis we used IBM SPSS *Statistics* 23.

RESULTS. Two groups of individuals were analysed: healthy and with CHD. Among 80 healthy adults 39 were women and 41 were men, with a median age of 36 and 41 years, respectively. In the case of self-reported CHD, 41 were women (median age 55 years) and 36 were men (age 56 years).

The T-C, LDL-C, ApoB and MMP-9 were statistically significantly lower in healthy individuals ($p < 0.0001$) compared with CHD patients. For healthy persons TG and hs-CRP also were lower but the difference was not so marked, being 0.016 and 0.026, respectively. The HDL-C and ApoA1 did not differ statistically between the groups.

We found that individuals with MMP-9 ≥ 53 ng/ml had 5 times higher risk to develop CHD (OR 5.09; 95% CI 2.27–11.39; $p < 0.001$). Furthermore, MMP-9 ROC analysis showed a statistically significant value in predicting CHD (AUC 0.77; 95% CI 0.69–0.85; $p < 0.001$).

Irrespective of age and gender, the IL-1β results were below 5 pg/ml, as the reference value recommended by the manufacturer.

CONCLUSIONS. The results of this investigation showed that elevated MMP-9 concentration can predict CHD and can be considered as a potential biomarker for CHD.

The study was supported by LITGEN Project (VP1-3.1-ŠMM-07-K-01-013).

PP-6. Serum index: first-year experience at Children`s Hospital

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OBJECTIVES. Pediatric patients, irrespective of their condition (physiological or pathological, such as dehydration, hypovolemia) are at an exeptionally high risk to provide a hemolysed blood sample. This requires additional costs and causes discomfort and stress to the little patient and his/her relatives. Hemolysed samples can be determined by the naked eye and can cause erroneous results. However, visual evaluation is subjective. The aim of this study was to evaluate the efficiency of a serum index program by analysing samples with an automated system.

METHODS. After examining sample hemolysis by the naked eye, 146 serum samples that were evaluated as hemolysed, were analysed with a Roche Cobas Integra 400 Plus analyser using the serum index application program.

RESULTS. A total of 349 hemolysis sensitive biochemical tests were performed for the 146 serum samples. The hemolysis index did not exceed the recommended individual hemoglobin concentration level for 242 out of 349 tests, enabling the performance of the biochemical tests. This accounted for 69% of all ordered tests.

CONCLUSIONS. Visual determination is not a reliable method of sample hemolysis. Automatized systems capable of determining the serum index results, based on test parameters, should be preferred to visual detection as a cheap and a fast way of accelerating and facilitating the standard laboratory process.

PP-7. Serum creatinine determined by the Jaffe and enzymatic methods in pediatric patients

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OBJECTIVES. The purpose of this study was to compare the serum creatinine concentration measured by the enzymatic method and by the Jaffe method, and to compare the effects of certain interfering substances such as glucose and bilirubin on creatinine levels measured in pediatric patients.

METHODS. Determination of serum creatinine concentrations in pediatric patients (with elevated bilirubin or glucose, n = 36, and control group, n = 25) was performed by the enzymatic method and by the Jaffe method using a Cobas Integra 400 Plus Roche analyser.

RESULTS. There was observed high correlation between the methods (r = 0.966, p < 0.0001). However, the coefficient of variation (CV %) and bias varied between the methods: Jaffe method – CV % 2.8%, bias – 7.9%, enzymatic method – CV % – 1.3%, bias – 1.0%. We observed significant positive interference due to glucose (14–45%) and significant negative interference due to bilirubin (28–38%) when the test was performed by the Jaffe method. When the test was performed by the enzymatic method, no interferences were found.

CONCLUSIONS. In conclusion, the enzymatic method is the best choice for determining serum creatinine levels in pediatric patients.

PP-8. Plasma corticosteroid analysis using a novel reversed-phase solid phase extraction sorbent that removes residual phospholipids

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BACKGROUND. Bioanalysis of drugs and endogenous molecules from plasma samples often involves using solid phase extraction (SPE) to clean up the matrix and concentrate the analytes of interest. Despite the advances in SPE sorbents and formats, residual phospholipids often remain in extracts and can interfere with analyses by causing ion suppression and by prematurely fouling analytical columns and MS sources. Using a novel SPE sorbent that is designed to remove phospholipids from samples, a panel of corticosteroids has been extracted from plasma. This sorbent is also water wettable, enabling extraction without the usual requisite preconditioning and equilibration steps. This has resulted in a method with excellent sensitivity that demonstrates consistent recovery, minimal matrix effects, and the elimination of > 95% of phospholipids compared to simple protein precipitation.

METHODS. 150 µl plasma samples were precipitated with 300 µl of a solution of MeOH and ZnSO4. After centrifugation, the supernatant was diluted with 4% H3PO4 and directly loaded onto a µElution SPE plate. All wells were then washed with 25% MeOH and eluted with 2 x 25 µl of 90:10 ACN:MeOH. The sample eluates were diluted with water and analyzed by UPLC/MS/MS.

RESULTS. All compounds eluted within 2 minutes. Recoveries were consistent, ranging from 72–73% with %CVs of less than 5%. Matrix effects were 19% for cortisol and less than 10% for androstenedione (Adione) and 17α-hydroxyprogesterone (17-OHP). Calibration curves were linear from 0.05–25 ng/ml for Adione and 17-OHP and from 1–500 pg/ml for cortisol, with R2 values of 0.99 or greater. %CV and bias values for quality control samples were less than 10% for all analytes at all QC concentrations. Comparison with samples that had been subjected to protein precipitation only revealed that 97% of phospholipids were removed by the SPE procedure.

CONCLUSION. The combination of water wettability and phospholipid removal has enabled the development of a simple, fast and clean extraction method that results in excellent reproducibility, minimal matrix effects and the elimination of residual phospholipids.

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PP-9. HCV genotypes circulating in Latvia in 2005–2015

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BACKGROUND. At present there are more than 40.000 patients with chronic hepatitis C in Latvia. Genotype (GT) and subtype determination of hepatitis C virus (HCV) is necessary in order to apply the most appropriate therapeutic regimen. At the same time HCV GT is predictive of the response to therapy.

AIM. To evaluate the prevalence of different HCV genotypes circulating in Latvia in 2005–2010 and 2011–2015 in the general HCV infected population and in patients with HIV/HCV coinfection.

MATERIALS AND METHODS. In this study there were included the genotyping results of 3209 patients treated for VHC in Latvia in 2005–2010 and 3063 patients treated in 2011–2015. Another group consisted of patients with HIV/HCV coinfection, with 62 and 69 samples, respectively. Genotyping was performed by LinearArray Test, Roche and Abbott RT HCV GenotypeII.

RESULTS. In the general population, in 2005–2010 as well as in 2011–2015, GT1 was predominant, at 64.4% and 62.5%, followed by GT3 at 32.3% and 33.8%, GT2 at 3.3% and 3.2%, GT4 at 1.6 % and 0%, respectively. Differences were found in the group of HIV/HCV coinfected patients. In the first period predominant was GT3 at 56.5%, followed by GT1at 38.7%, in the later period the situation was the opposite (GT1 at 50.7%, GT3 at 47.8%).

In the predominating GT1 subtype 1b was detected more often, in 74%, while GT1 subtype 1a was only detected in 3.8%. The high proportion of GT1, 21.9 %, was not differentiated by subtypes.

CONCLUSION. The relative prevalence of different genotypes in the general chronic VHC population remained stable over time (GT1, 64.4% – 62.5%; GT3, 32.3% – 33.8%). In the predominating GT1 subtype 1b was detected more often, which is in accordance with previously published data for Latvia. Changes in the proportions of GT1 and GT3 in the group of HIV/HCV coinfected may be explained by decreasing of IDU in the population of HIV infected persons in recent years.

PP-10. Distribution of HLA B27 allele in Estonian patients with spondyloarthropathies

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OBJECTIVES. The association between HLA B27 and spondyloarthropathies is well known for a long time. However, the degree of association between HLA B27 and spondyloarthropathies varies markedly in different forms of spondyloarthropathies and among different populations worldwide. To date, no data are available about the frequency of HLA B27 in patients with spondyloarthropathies in our country. The aim of this study was to determine retrospectively the distribution of the HLA B27 allele by different types of spondyloarthropathies in patients attending our clinic.

METHODS. The study included 212 patients admitted to the department of rheumatology of East-Tallinn Central Hospital who were diagnosed with different spondyloarthropathies. The patients were divided into four groups: ankylosing spondylitis (AS, n = 58), reactive arthropathies (RA, n = 65), psoriatic spondylitis (Ps, n = 10) and undifferentiated inflammatory spondylopathies (Usp, n = 79). Patients with seropositive rheumatoidarthritis served as the control group (n = 81). The HLA B27 allele was tested using a commercial real-time PCR assay. The frequency of the HLA B27 allele was calculated for the different groups.

RESULTS. A significantly higher frequency of the HLA B27 allele in patients with spondyloarthropathies was found compared with the control group, 45.2% vs. 13.6% ($p < 0.0001$). The highest frequency was observed in AS patients, at 86.2%. In the RA group the frequency of the HLA B27 allele was 26.2%, in Ps 30% and in 32.9% in Usp.

CONCLUSIONS. Our study confirmed the association of HLA B27 with spondyloarthropathies, and the importance of HLAB27 testing as an aid to diagnosis of spondyloarthropathies, especially in AS. The frequency of HLA B27 in our study group of AS was similar to that found in some Central and South European countries but lower than that reported from Northern European countries.

PP-11. Diagnostics of tick-borne encephalitis virus infection from acute antibody negative samples by real-time PCR

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Tick-borne encephalitis virus (TBEV) is an RNA flavivirus. The infection of humans occurs via bite of an tick while the central nervous system (CNS) can be affected. The first phase of the disease manifests through flu-like symptoms and viraemia occurs simultaneously. No antibodies are detectable at this stage. After an afebrile period of 2–10 days, seroconversion can be observed and detection of IgM and IgG by ELISA becomes possible. Thus the main problem of the serology based diagnostics, which is widely applied at present, is that the infection can be reavealed only during the second phase when the symptoms are severe and CNS is already involved. Moreover, since the viraemic phase is very short and appears simultaneously with unspecific symptoms, RNA detection is difficult.

AIM. To detect RNA of TBEV in early patient serum samples. Paired sera of 54 acute TBE patients were analysed. The first sera, taken immediately after the patient presented at hospital, were screened for TBEV specific IgM and all samples were further analysed by real-time PCR. For all 54 patients, the infection was confirmed by detection of TBEV specific IgM and IgG in the second- phase serum, taken approximately 2 weeks after the first one.

RESULTS. Out of 33 IgM negative first phase sera, 24 had detectable levels of TBEV RNA. The presence of RNA was also demonstrated in 3 out of 5 samples that were on the borderline of IgM negativity and positivity. Finally no viral RNA was found in any of the IgM positive samples. Out of 27 RNA positive samples 19 were successfully amplified and sequenced either in the complete E gene or partial E gene region.

CONCLUSION. IgM negativity might correlate with RNA positivity and real-time RT-PCR can be a useful tool for early diagnostics of TBE. Real-time PCR analysis of samples from patients with a known tick bite history or from those who have been to a TBEV endemic area could be of benefit.

PP-12. Comparison of hyaladherin expression on the surface of T lymphocytes in BAL fluid and peripheral blood samples from patients with pulmonary sarcoidosis

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OBJECTIVES. It is known that in about 60% of cases the course of sarcoidosis is self-limiting with spontaneous resolution of the granuloma. Nevertheless, for other patients the disease can progress leading to fibrosis with organ failure. Some clinical studies show that correlation exists between increased levels of hyaladherin (CD44) in BAL and various interstitial lung diseases. We hypothesize that alveolar CD44 positive T cells may play a role in sarcoidosis pathology, too.

METHODS. We analysed T cell subpopulations (CD4+CD44+ and CD8+CD44+) in BAL and peripheral blood (PB) samples of patients (Pts) with lung sarcoidosis and in healthy controls (H): 67 Pts (mean age 40 ± 12 years) and 12 H (mean age 40 ± 8 years). The BAL and peripheral blood samples were analysed by flow cytometry (BD FACSCalibur) using three colour monoclonal antibodies to cell surface antigens.

RESULTS. Our study revealed significant differences in the $CD3^+CD4^+CD44^+$ cell number between Pts and H BAL (75.9 ± 13.5; 47.2 ± 14.6, respectively) and between Pts BAL and PB (75.9 ± 13.5; 45.8 ± 9.5, respectively). At the same time, the number of BAL $CD3^+CD8^+CD44^+$ cells did not differ significantly between Pts and H (20.7 ± 12.7; 37.6 ± 14.6, respectively) or between Pts BAL and PB (20.7 ± 12.7; 38.6 ± 10.8, respectively).

CONCLUSIONS. According to our investigation, CD3+CD4+CD44+ cells are playing a major role in sarcoidosis inflammation; we can conclude that these lymphocytes are implicated in the immunophatological mechanisms of sarcoidosis. Measurement of CD3+CD4+CD44+ lymphocytes in BAL can be used in determining the activity of sarcoidosis and in predicting the disease course.

PP-13. Implementation of high sensitivity architect troponin-I assay in routine practice: advantages and challenges

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OBJECTIVES. The first cardiac-specific Troponin I (cTnI) assay was described in 1987 by Cummins et al. Further development of TnI assays led to development of high sensitivity TnI (hsTnI) assays that lower detection level, which allows to evaluate heart attack within two to four hours. Abbott recently introduced the hsTnI assay for Architect i analysers. The aim of this study was to compare the new hsTnI assay with the conventional TnI assay.

METHODS. We analysed cTnI concentration in the serum samples of 467 unselected patients (183 female and 284 male) admitted to the Emergency Department. The cTnI concentrations were measured with the Abbott Architect i2000 analyser (Abbott Diagnostics, USA) using two immunoassays: STAT hsTnI and STAT TnI. Passing-Bablok regression analysis was used for comparison of the methods.

RESULTS. In 45 results out of 467, the ng/l value with the conventional TnI assay was 0, while with hsTnI it ranged between 0.1–12.9 ng/l with a mean of 2.95 ng/l. We determined hsTnI within-run precision using 72 serum samples at the 99 percentile level for both males and females and average CV = 3.37% was calculated. We found poor agreement for the normal and pathological female groups: intercept: 1.05 with 95% confidence interval (0.45 to 1.91), slope: 0.6 (0.45 to 0.8); intercept: -7.33 (-12.78 to -1.06), slope: 0.95 (0.91 to 0.98), respectively. The normal and pathological male groups demonstrated a similar situation: intercept 0.5 with 95% confidence interval (0.1 to 1.04), slope: 0.68 (0.62 to 0.76); intercept: -17.66 (-28.87 to -8.94), slope: 0.9995 (0.95 to 1.03), respectively.

CONCLUSION. The hsTnI showed higher sensitivity in the lower range of cTnI concentration and excellent precision (CV far below 10%) at the levels of 99th percentiles in both the male and female groups. The hsTnI identifies a larger number of patients with myocardial injury and requires different approaches in clinical decision making. There were found systematic and proportional differences between the two methods.

PP-14. Influx of Syrian refugees and cutaneous ceishmaniasis in the city of Adana, Turkey

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OBJECTIVES. More than four million refugees of the Syrian civil war have left the country during the course of the war. Most of them fled to neighboring Turkey. As of December 2015, Turkey was the world's biggest refugee hosting country with close to 2.5 million Syrian refugees. Some of them reside in Adana, southern part of Turkey. One of four immigrants lives in the southern part of Turkey. Cutaneous leishmaniasis represents an alarming problem for the whole area of the Middle East. Currently, an outbreak has been observed due to the war in Syria and the lack of measures to combat the disease, particularly in the besieged and medically underserved areas. The aim of the study was to draw attention to the dramatic increase in the number of new cases of cutaneous leishmaniasis in Adana, after the beginning of the civil war in Syria.

METHODS. Between January 2014 and January 2016, a total of 113 smear samples were taken from cutaneous leishmaniasis suspected Syrian refugee cases and sent to the Department of Parasitology Laboratory, Faculty of Medicine, University of Cukurova, for molecular analysis. The samples were analysed with real-time PCR.

RESULTS. Fourty two (37.2%) smear samples were positive for cutaneous leishmaniasis according to the real-time PCR method. Three different Leishmania species were found in the 42 cutaneous leishmaniasis cases by real-time PCR: 39.6% *Leishmania tropica*, 37.1% *Leishmania infantum* and 23.3% *Leishmania major*.

The results of real-time PCR were confirmed with Leishmania ITS1 DNA sequencing.

CONCLUSIONS. This study revealed that in Adana, southern part of Turkey, Syrian refugees are an important problem in terms of transmission of cutaneous leishmaniasis. Refugees in Turkey, and also in Europe, pose a risk, given the availability of vectors, for the transmission of the disease to new, as yet unaffected countries.

PP-15. Genotyping of hepatitis C viruses for selecting the dosage of treatment

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OBJECTIVES. Accurate determination of HCV genotype is important in the management of patients' anti-HCV treatment. The hepatitis C viral genome is highly variable and is classified into 6 genotype groups, or clades, based on phylogenetic analysis of the genomic sequence. These genotype groups differ in 31% to 34% of their nucleotide sequence positions and in about 30% of their amino acid sequence positions.

We detected HCV RNA and determined the amount of viral RNA for 133 primary patients at Klaipeda University Hospital. As a result, 95 out of them were positive for HCV RNA and the genotypes of HCV were evaluated.

Samples: human EDTA plasma from 133 patients.

METHODS. Molecular analysis: antiHCV testing was performed for ECLIA Elecsys® Anti-HCV II, Cobas, Roche; quantitative detection of HCV specific RNA was performed using real-time RT-PCR *artus* HCV RG RT-PCR Kit, Qiagene; and HCV genotyping was performed using VERSANT® HCV Genotype 2.0 Assay (LiPA), Siemens.

RESULTS. In our analysis 95 out of 133 samples from patients were positive for HCV. Genotype 1 was detected in 62 samples (1a for 8, 1b for 44, others – unspecified subtype of genotype 1), one sample revealed genotype 2 and genotype 3a was detected in 32 samples. Anti HCV testing was performed for 30% of the patients and subtenant results were controversial.

CONCLUSIONS. The most prevalent HCV genotype for our HCV patients was genotype 1, 1b subtype. Determination of the HCV genotype is important when selecting an optimal dose of interferon and ribavirin and duration of treatment.

PP-16. The role of client-initiated laboratory testing in discovering hidden diseases at East-Tallinn Central Hospital

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BACKGROUND. East Tallinn Central Hospital offers two sets of selected analyses, commented by laboratory doctors, for persons who are interested in checking their health without a doctor's referral. The set Primary laboratory analyses (PLA) consists of 9 tests (19 parameters) and written comments; the set The first step for health estimation (SHE) consists of 17 tests (26 parameters) and a laboratory doctor's consultation.

OBJECTIVE. To describe clients' characteristics and test results' deviations for the PLA and SHE sets from August 2014 to December 2015.

METHODS. Data were retrieved from the hospital's electronic database. The clients' characteristics, and 19 parameters (CBC-Diff and urine strip test, Gluc, TSH, ALAT, GGT, Crea, eGFR, CRP, Chol) for the PLA set and 26 parameters (Trigl, HDL-Chol, LDL-Chol, IgE, HCV Ab, HBsAg, St-Hb, PSA (male) in addition to the foregoing) for the SHE set were described.

RESULTS. The PLA was selected by 108 clients (65% female, mean age 44 years; PLA-clients) and SHE was selected by 109 clients (57% female, mean age 46 years; SHE-clients). Forty-nine SHE-clients had complaints (not estimated for PLA).

At least one test result remained outside its reference limits (RL) for 90% of the clients. The biggest number of result deviations per client was 15/25 parameters for a SHE-client. Of all pathologic tests, 38% indicated problems of cholesterol metabolism; the results of 59% LDL-Chol, 52% Chol and 44% HDL-Chol tests remained outside the RL. More than of 15% the results were outside the RL also for blood glucose, creatinine and IgE. There were found no new positive HCV-Ab or HBsAg cases.

Five clients with previously undiagnosed health problems were referred to specialists. The clients with slight deviation in test results were recommended to repeat analyses or to visit a family doctor.

CONCLUSIONS. Client-initiated laboratory testing accompanied with laboratory doctors' comments helps to discover hidden health problems. Analysis of the results allows to improve the selection of tests to be offered.

PP-17. Mosaic *de novo* tetrasomy of 5q35 in a newborn with typical features of 5q35 duplication syndrome and heart defect

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Recently described 5q35 microduplication reciprocal to common Sotos syndrome deletion (~2 Mb) is associated with microcephaly, short stature, developmental delay and delayed bone maturation. The clinical picture is largely opposite to that of Sotos syndrome (macrocephaly, overgrowth and advanced bone age). The dosage effect of *NSD1* (5q35) is suggested to be the cause of main clinical problems.

We report a dysmorphic newborn with IUGR, microcephaly, short stature, and prenatally diagnosed congenital heart defect (ASD, VSD, left ventricle hypoplasia). First routine karyotype analysis (GTG-banding) revealed a normal male karyotype. Chromosomal microarray analysis (CMA, HumanCytoSNP-12 BeadChip, Illumina Inc.) revealed a ~ 8.3 Mb duplication of 5q35.1-qter. To localize the extra copy, FISH analysis was made with Kreatech hTERT/NSD1 and 5p/5q subtelomeric probes. Surprisingly, two additional signals of NSD1 and 5qter were seen, located on a small additional marker chromosome, which was present in ~ 10% of metaphases (all of them were of low band quality and had thus escaped first analysis), and in 50% of interphase nuclei.

Mosaic tetrasomy due to presence of der(5q) marker-chromosome in ~ 50% of cells results in a typical duplication picture seen in CMA, as well as in clinical features typical of 5q35 duplication. The more severe clinical picture compared to common 5q35 duplication can be explained by encompassing NK2 (cardiac development) and MSX2 (limb and bone development) genes.

PP-18. Comparison of blood sample transport via pneumatic tube system and manually by courier

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OBJECTIVES. It has been recommended that each hospital investigates its own pneumatic tube system (PTS) for susceptibility to haemolysis. Our aim was to compare haemolysis indices (HI), concentration of cellular and biochemical components and sample delivery time between the Swisslog PTS and courier transport.

METHODS. We verified the Swisslog PTS between the blood taking unit and laboratory reception. Duplicate samples were collected for four different sample types (EDTA, serum, Na-citrate and Li-heparin, each n = 20), sent to the laboratory through the PTS (speed 6–8 m/s) and delivered by a laboratory assistant. Haemolysis indices were measured spectrophotometrically (Cobas 6000, Roche Diagnostics). Selected tests were analysed (white blood cells, platelets, INR, potassium). Differences between the pairs of samples were evaluated with a paired t test or a sign test (in case distribution was not normal). A p-value of ≤ 0.05 was considered significant.

RESULTS. There was no significant difference in the HI, leucocyte, platelet or INR values between the samples sent via the PTS or those delivered manually ($p > 0.05$). Mean potassium values with the 95% confidence interval were 4.29 (4.11–4.46) for the PTS and 4.20 (4.02–4.37) mmol/l for courier transport ($p = 0.0078$). However, there was no significant difference in HI for these potassium samples. The geometric mean delivery time of the samples was 23 (20–27) min by the courier and 17 (14–20) min via the PTS ($p = 0.0045$, $n = 80$).

CONCLUSION. Transport of blood samples via the PTS is faster and does not influence results to a higher degree than routine transport by the courier. Higher potassium values cannot be explained by possible haemolysis induced by PTS.

PP-19. Prevalence of non-tuberculous mycobacterium strains isolated from clinical specimens at North Estonia Medical Centre 2001-2015

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Mycobacteria are widespread microbes in the nature. At present more than 120 different species of mycobacteria are known. Mycobacteria that are causative agents of human and animal tuberculosis are called the Mycobacterium tuberculosis complex (MTBC). Some of mycobacteria that are not MTBC have been considered to be named non-tuberculous mycobacteria (NTM). The diseases caused by NTB are called mycobacteriosis.

OBJECTIVES. The incidence of tuberculosis in Estonia shows a trend of decrease. The aim of the study was to analyse the prevalence of NTM strains on the basis of clinical specimens.

METHODS. The NTM species were isolated from clinical material that had been sent to the mycobacteriology laboratory for diagnostic purposes during 2001–2015.

The pathological material was decontaminated by NaOH+NALC and cultivated on Löwenstein-Jensen and Middlebrook. The NTM was identified by the GenoType Mycobacterium CM/AS test (Hain Lifescience GmbH).

RESULTS. In 2001–2015, of 106,820 investigated specimens, 12,881 (12%) MTBC and 880 (0.82%) NTM strains were identified.

The leading NTM species isolated were *M. avium* (48%), *M. gordonae* (12%), *M. fortuitum* (10%) and *M.intracellulare* (5%). Among rarely isolated NTM there were *M.kansasii*, *M.xenopy*, *M.szulgai* and *M.abscessus*. Of the NTM, 72% were cultivated from sputum, 5% from blood and 23% from other materials.

The number of patients infected with NTM was 85 in 2001–2005, 210 in 2006-2010 and 237 in 2011–2015.

Of the patients releasing NTM, 35% were older than 65 and 9% were younger than 30 years of age. A combination of TBC and HIV was found in 317 (8.3%) and a combination of NTM and HIV was found in 56 (10.5%) patients.

CONCLUSIONS. During the last 15 years the prevalence of NTM in patients' material has increased approximately three times. The most prevalent species is *M. avium*.

PP-20. Peculiarities of lipid metabolism parameters for cardiovascular disease risk assessment

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INTRODUCTION. In diagnostics of cardiovascular disease it is necessary to pay attention to age, gender, concentrations of total cholesterol (TChol), high density lipoprotein (HDL), low density lipoprotein (LDL) choltesterols, triglicerides (Tg) and other risk factors. A significant diagnostic value in assessing the condition of the patient can be ascribed to lipid relative values: TChol/HDL, LDL/HDL and Tg/DTL.

MATERIALS AND METHODS. Analysis of the test results of 876 patients (443 male and 433 female) with an average age 59.5 ± 15.6, with cardiovascular, chronic liver, renal and hormonal imbalance diseases, who were treated at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics. The patients were divided into 3 age groups (up to 50, 50–65 and over 65 years). The lipid profile parameters (TChol, HDL, LDL cholesterols and Tg), blood analysis, relative values (TChol/HDL, LDL/HDL, Tg/HDL) and atherogenic factor (AF) were calculated. Statistical analysis was performed using the IBM SPSS 20.0 statistical data analysis package. The result was statistically significant when the probability of error was $p < 0.05$.

RESULTS. Women aged 50–65 years with chronic liver, renal and hormonal imbalance diseases were found to have the lowest lipid profile parameters, while men with cardiovascular diseases were found to have the highest values of AF and lipid ratios ($p < 0.05$). Lipid metabolism parameters for older patients than 65 years were found to be better compared with those for patients younger than 50 years, especially in the group of men with cardiovascular disease ($p < 0.05$).

CONCLUSIONS. Women aged 50–60 years with a chronic disease can be assigned an increased risk of developing cardiovascular disease.

PP-21. Screening for EGFR, RAS and BRAF genes mutation in NSCLC, MCRC and melanoma specimens

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OBJECTIVES. The rapid development of targeted therapies has tremendously changed the clinical management of setting the stage for a number of tumour types. Evidence has accumulated that targetable drugs show the best efficacy and improve progression of survival rates. For cancer patients whose tumours have a specific genotype, molecular testing for predictors of therapy response has become the standard of care. At present, for selecting appropriate target therapy, testing for *EGFR* mutations in lung adenocarcinoma, *RAS* mutations in mCRC, and *BRAF* mutations in melanoma is carried out in oncology practice.

We performed predictive testing for the mutation status of *EGFR*, *RAS* and *BRAF* genes for patients with metastatic lung, CTC and melanoma patients from FFPI material at Klaipeda University Hospital, Lithuania.

METHODS. Samples: formalin-fixed tumour samples were obtained from and diagnosed for 152 patients at Klaipeda University Hospital, Lithuania.

Molecular analysis: mutation test for qualitative detection and identification of mutations therascreen *EGFR* Pyro Kit in exons 18, 19, 20 and 21 of the *EGFR* gene, therascreen KRAS, *NRAS* Pyro Kit (hot-spot) and therascreen *BRAF* Pyro Kit V600 codon in DNA derived from formalin-fixed paraffin-embedded (FFPE) human tumour tissue.

RESULTS. Altogether 71 DNA samples from NSCLC patients were tested for *EGFR* mutations and 13 cases (18.3%) out of 71 were *EGFR* mutated.

Sixty DNA samples from mCRC patients were tested for RAS mutations, 33 cases (55%) were RAS mutated.

Twenty-one DNA samples from 21 mCRC patients were tested for *BRAF* mutations, 11 cases (52%) were BRAF mutated.

CONCLUSIONS. An up to date concept of personalized therapy for NSCLC, CTC and melanoma involves characterization of individual patient's tumour mutation status.

Rapid molecular results are essential for diagnosis of advanced malignant tumour because of the urgent need to initiate the most appropriate therapy for a certain disease type.

PP-22. The effect of physical training on platelet aggregation and fibrinogen concentration in patients with chronic heart failure

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OBJECTIVES. Data about the importance of physical load on hemostasis in patients with chronic heart failure (CHF) are controversial. The aim of the study was to find out the effects of long-term physical load on fibrinogen concentration and platelet aggregation.

METHODS. Platelet aggregation and fibrinogen concentration were investigated in 144 patients. The trained subjects and the controls were investigated as follows: on admission to hospital, after treatment at hospital, after 6 months, after 1 year. The indices were investigated before and after physical load.

RESULTS. Fibrinogen concentration increased significantly after the application of physical load in all investigated stages for both groups ($p < 0.045$). In the course of treatment, fibrinogen concentration gradually decreased in the trained subjects ($p < 0.02$) in all investigated stages. Platelet aggregation investigated with ADP increased significantly after physical load in all stages for both groups ($p < 0.001$ and $p < 0.045$, respectively) and decreased during the different stages of investigation for the groups of the untrained ($p < 0.02$) and trained subjects. Platelet aggregation investigated with ADR decreased consistently before applying physical load during the different stages of investigation for the groups of the trained subjects and untrained patients ($p < 0.02$).

CONCLUSIONS. Physical training reduces fibrinogen concentration in patients with CHF. It remains unclear whether physical training can have an effect on decrease in platelet aggregation in patients who have been engaged in long-term physical training.

PP-23. Results of the effect of hypochlorous acid on platelet aggregation in healthy subjects and in patients with heart failure in vitro

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OBJECTIVES. It is important to explore the impact of oxidants on platelet aggregation as this might serve as a niche for treatment. The aim of this study was to determine the dependence of the effects of hypoclorous acid on the intensity of platelet aggregation in vitro in healthy subjects and on HOCL concentration in heart failure patients.

METHODS. Were investigated: healthy subjects (n = 10) and heart failure patients (n = 18). Platelet aggregation was determined with an optical 2-channeled Chrono-log platelet aggregometer. Aggregation was initiated with ADP (3.8 mmol/l) solution. We used 6 samples from the same person`s plasma. To each sample 10 µl HOCl of different concentrations were added: 2.12 mm/l, 7.06 mm/l, 10.59 mm/l, 21.18 mm/l, and 43.4 mm/l being final concentration. To the control sample the same volume of physiological solution was added. The samples were incubated for 30 min at 37 ^{0}C, and the intensity of platelet aggregation using ADP was determined (%).

RESULTS. It was found that high concentrations of HOCl (2.12 to 43.4 mm/l) significantly decreased platelet aggregation in both groups. The increasing concentration of HOCl was reversely proportional to the intensity of platelet aggregation. The decrease of platelet aggregation intensity in the plasma of patients affected with HOCl *in vitro* was more pronounced compared with healthy subjects (42.6% and 36.1%, respectively).

CONCLUSIONS. The intensity of platelet aggregation significantly decreased depending on HOCl concentration in plasma *in vitro*. Heart failure patients were more affected than healthy subjects.

PP-24. Prevalence of single nucleotide polymorphisms RS12979860 and RS8099917 of the interleukin 28B gene among hepatitis C virus positive patients at East-Tallinn Central Hospital

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OBJECTIVES. The natural course of hepatitis C virus (HCV) and response to therapy depends on viral factors and host factors (age, sex, liver fibrosis etc). Additionally, there is a new host component – two single nucleotide polymorphisms (SNPs) near the interleukin 28B (IL28B) gene. The SNP rs12979860 genotype CC and the rs8099917 genotype TT are associated with increased spontaneous clearance of the HCV genotype 1 and are also predictors of sustained virologic response (SVR) following pegylated interferon-α and ribavirin (PEG-IFNα/RBV) therapy. The aim of the present study was to estimate retrospectively the genotype frequency of IL28B SNPs in HCV-infected patients at East-Tallinn Central Hospital.

METHODS. Genomic DNA was isolated from whole blood samples collected from 98 HCV infected patients. Detection of polymorphisms rs12979860 and rs8099917 was performed with commercial real-time PCR kits using a Rotor Gene Q thermal cycler.

RESULTS. Frequencies of the IL28B 2 SNP genotypes were determined in 98 HCV-infected patients. The favourite genotype CC/TT (rs12979860/rs8099917) was detected in 19.4% of the cases, the unfavourite genotypes CT/TG and TT/GG, in 44.9% and 3.1% of the cases, respectively.

Predominantly, there were carriers of the rs12979860 CT genotype (64/98, 65%). The frequency of the favourite rs12979860 C-allele was 52%.

CONCLUSIONS. The results indicate that IL28B SNP genotyping may be an effective tool for physicians with patient management predicting the likelihood of HCV response to therapy. The selection of our study group explains lower rs12979860 C-allele frequency compared with overall population frequencies.

PP-25. Intracellular neutrophil myeloperoxidase in pediatric patients during chemotherapy

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Leukopenia is life-threatening in pediatric patients undergoing chemotherapy; still, there are few criteria for predicting its severity and outcome. Neutrophil myeloperoxidase (MPO) is produced in azurophilic granules from the earliest stages through maturation and is excreted in activation and phagocytosis. The analyser Advia 2120i measures MPO to separate myeloid cells as Myeloperoxidase Index (MPXi, range -10–+10).

OBJECTIVES. The aim was to analyse episodes of chemotherapy-induced leukopenia in children for dynamics of leucocyte count (WBC) and MPXi. The data for 2011–2013 from the Children's Clinical University Hospital LIS were selected. Altogether 336 episodes of leukopenia were found; 294 representative cycles of 85 patients with a total of 3824 blood tests were studied. The MS Excel and IBM SPSS v.21 were used for statistical analysis.

RESULTS. In 283 cycles (96%) there was a synchronous drop in WBC and an increase of MPXi above +10, followed by synchronous normalization. In 5 cycles (2%) MPXi did not reach +10. Irregular MPXi fluctuations were seen in 6 cycles (2%). WBC and MPXi reached their peaks simultaneously (median difference 0 days), MPXi reverted to normal earlier (median 1 day, the difference significant, $p << 0.0001$).

Negative correlations between MPXi and WBC and neutropenia grade were found ($p << 0.0001$ for both). Duration of leukopenia correlated with time span of MPXi being > +10, with increase in MPXi from initial through peak and with maximal attained MPXi ($p < 0.001$ for all).

CONCLUSIONS. The results strongly support the relationship between destruction and restitution of neutrophils during chemotherapy and their MPO content. In 98% leukopenia cycles, cellular MPO increased synchronously with a decrease in WBC and decreased along WBC recovery. Thus, cellular MPO could be used as a predictive factor in induced leukopenia. The MPXi by Advia 2120i is excellent for monitoring since it can be measured during routine blood tests.

The results of the study are completely original, there are no available comparable published data.

PP-26. Red cell indices and C-reactive protein in pediatric patients are closely related

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Disease-related anemia in children, though a common knowledge, is poorly described; only sporadic historical data, studies on small hospital cohorts or in general population are available in the literature.

OBJECTIVES. The aim was to correlate blood red cell indices (HGB, RBC, MCV, MCHC) with CRP in pediatric patients in order to analyse relations between anemia and inflammation.

METHODS. 70024 routine blood tests (Advia 2120i) with parallel CRP (Cobas 60000) performed at Riga Children's Clinical University Hospital in 2011–2013 were analysed. Results from the Emergency, Neonatology, Intensive Care, Hematooncology, Infections and Outpatient departments were further studied. Statistics was performed by MS Excel and IBM SPSS v21 (Spearman rho for correlations and Mann-Whitney U for differences).

RESULTS. Of the samples 27.8% were anemic after age and gender adjustment, 11.7% of them were possibly iron-deficient (MCHC low, MCV low/normal); only 0.3% had both high MCV and MCHC. The HGB, RBC and MCV significantly correlated with CRP for the total cohort ($p << 0.0001$). The HGB was significantly lower in patients with CRP 5-50 than in patients with normal CRP for all groups but Neonatology; and significantly lower in patients with CRP > 50 than in patients with CRP 5-50 for all groups. The CRP was significantly elevated in patients with anemia ($p << 0.0001$ for the total cohort and for all groups).

The RBC count was elevated in 3.5% of the tests after age and gender adjustment. Paradoxically, it was associated with a significantly lower CRP for the total cohort, and for Emergency, Intensive Care and Infections.

CONCLUSIONS. The study was performed on a much broader basis compare with previous reports. The results support observations of frequent anemia in a pediatric hospital setting, mostly not iron-deficient. The HGB and CRP were highly significantly related for the whole cohort and for the specific patient profiles. No studies of occasionally found erythrocytosis have been published; the finding of plethora associating with lower CRP needs further analysis.

PP-27. Comparison of two procalcitonin and C-reactive protein testing methods

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INTRODUCTION. A differential diagnosis of infection caused by either bacteria or other microbial organisms is essential for effective treatment and prognostic assessment. Current clinical laboratory methods in the diagnosis of bacterial infections (incl. sepsis) are either non-specific or require longer turnaround times. Procalcitonin (PCT) and C-reactive protein (CRP) are biomarkers of bacterial infection with distinct clinical qualities, and they can be used in parallel to improve the specificity and sensitivity of the diagnosis of bacterial infection / sepsis.

There is currently no agreement-based reference method for PCT and CRP testing, but all laboratory methods for quantification of PCT and CRP are based on immunoassays.

AIM. To compare the PCT and CRP results measured with the Roche Cobas and hybcell automated assays.

MATERIAL AND METHODS. 12 clinical samples from a Sepsis clinic were tested with two different PCT and CRP testing methods – *hybcell Inflammation Blood xA* (hybcell technology based on cylindrical microarrays) and *PCT BRAHMS* and *CRP-Latex* immunoturbidimetric automated assays (Roche). Devices used: hyborg Dx Red (Cube Dx GmbH, Roche Cobas Integra 400plus, Roche Cobas e411.

The study was launched on 01.01.2016. Clinical samples were selected to cover clinically significant areas: PCT negative and elevated, PCT that predicts sepsis, CRP within the reference range and elevated.

Deming regression analysis and R^2 were used to compare the results.

PRELIMINARY RESULTS. Absolute values of *hybcell* CRP results correlated with those obtained with *Roche Cobas*, with $R^2 = 0.6423$. This correlation is acceptable.

The absolute values of *hybcell* PCT results correlated with those obtained with *Roche Cobas*, with $R^2 = 0.98$. This correlation is very good, however, there was noted a shift in absolute values around factor 5.

CONCLUSIONS. In general, there is good correlation of *hybcell* and *Roche Cobas* values for both parameters.

The reason for a shift in absolute PCT values can be explained with the use of different antibodies which bind to different parts of the PCT molecule: in the case of PCT BRAHMS it detects the intact CT/CCP protein, but *hybcell* detects N-PCT/CT. If PCT molecule in patient`s serum is present undigested, there can be expected similar results with both systems.

Further evaluation of both testing methods should be performed to evaluate clinical outcome as well as to give recommendations to clinicians for interpretation of results.

PP-28. Research of enterobiaisis among nursery school children in four Estonian counties

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INTRODUCTION. Since 2002 the Tartu Health Care College has researched enterobiasis in Estonian nurseries. As approximately ten years have passed since the first studies, there is reason to find out if infection rate among children has decreased. The aim of the research was to find out the distribution of pinworms and conditions influencing the distribution in four Estonian counties.

METHODS. The research was conducted in Jõgeva, Tartu, Viljandi and Rapla counties. During 2012–2015, altogether 1322 children were examined in 38 nurseries. For examination, the traditional anal swab method was used. Questionnaires were collected only from the parents whose children provided anal swabs; altogether 1102 completed questionnaires were returned. The questionnaires included questions about home environment and parent's awareness of pinworms. Interviews with teachers included questions about conditions at the nursery.

RESULTS. Altogether 259 (19.6%) children were infected with pinworms on the basis of the single examination, infection rate varied by counties. Infection rate was slightly higher among boys, at 21.6%, compared to girls, at 17.2% ($p < 0.05$). Infection rate depended on child's age ($p < 0.05$). Children's infection is related to their mothers' level of education. The highest infection rate occurred among children whose mothers had primary education (rate 27.9%) and the lowest rate occurred among the children whose mothers had higher education (11.8%, $p < 0.05$).

CONCLUSION. Compared to the research conducted in several Estonian counties in 2002–2007 the infection rate has not markedly decreased. Even though there is relationship between mothers' level of education and children's rate of infection, it seems that the level of education is not connected with the awareness of enterobiasis. Further research is needed concerning the relationship between fathers' level of education and infection rate and awareness of enterobiasis among nursery school teachers.

PP-29. Specific proteinase-3 and myeloperoxidase antibody rate among patients with positive anti-neutrophil cytoplasmic antibody results

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OBJECTIVE. Autoantibodies against neutrophil cytoplasmic antigens (ANCA), which react with the proteolytic neutrophil granule enzymes, proteinase-3 (PR3) and myeloperoxidase (MPO), have been found in patients suffering from various forms of autoimmune vasculitis. The aim of this research was to identify and evaluate specific PR3 and MPO antibody rate among ANCA positive patients depending on gender and age.

METHODOLOGY. Altogether 78 ANCA positive patients were analysed. Additionally, the ANCA cases were studied by selecting a qualitative indirect immunofluorescence method using EUROPLUS Granulocyte Mosaic 25, Germany. By assessing the fluorescence type of antibody in formalin and methanol and reactions with PR-3 and MPO antigens, ANCA was divided into cANCA, typical and atypical pANCA. Statistical analysis of the data was performed using the IBM SPSS 20 program.

RESULTS. Twenty-nine (37.2%) of 78 ANCA positive patients were found to be typical pANCA positive, 11 (14.1%) were found to be atypical pANCA, 14 (17.9%), cANCA and 24 (30.8%), ANCA negative. The specific antibodies against PR3 were found in all 14 subjects with a cANCA positive; specific antibodies against MPO were found in 17 (58.6%) of 29 patients with typical pANCA positive. In 54 women participating in this research the most frequent were typical pANCA (41%), less frequent were atypical pANCA (15%) and cANCA (13%). For 24 tested men the frequency of pANCA and cANCA was distributed equally, i.e. by 29%. Assessing the results by age, the atypical pANCA were more often found in older women than in men (73.3 ± 8.8 and 49 ± 21.9, respectively), while cANCA was found in both younger women and younger men (61 ± 13.8 and 45.6 ± 12.9, respectively).

CONCLUSIONS. Typical pANCA are more commonly found in women, while pANCA and cANCA are equally found in men, and atypical pANCA are found in older women and younger men. For the interpretation of ANCA results, additional investigations of antibodies against MPO and PR3 antigens would provide complementary diagnostic information and help to diagnose, predict and monitor the progress of vasculitis more accurately.

PP-30. Acquired cytogenetic finding in a patient with acute myeloid leukemia

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Acute myeloid leukemia (AML) is a disease resulting from the clonal expansion of myeloid blasts in the peripheral blood, bone marrow or other tissues. Clinically, morphologically and genetically it is a heterogeneous disease and may involve only one or all myeloid lineages.

Cytogenetic analysis is an integral part of the diagnosis and management of patients with AML to identify a series of recurrent genetic markers by which AML is subcategorised.

The aim of the study was to analyse the results of cytogenetic examination carried out among 123 patients of different Caucasian populations with AML during 2006–2015 at the E. Gulbis laboratory in Latvia.

Cytogenetic analysis was performed from cultured cells of bone marrow or peripheral blood without stimulation, using the GTG banding method, and was karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). The FISH method was used in the case of need to determine cryptical changes or to confirm discovered ones.

Chromosome analysis in a patient with AML helped to detect structural and numerical abnormalities in 61 (49%) samples. The group of the most significant changes was t(8;21) or its variations (7%); changes of 7.chromosome were found in 6% of the samples; t(15;17) were found in 6%; 5.chromosome changes, in 5%, including complex changes; 8.chromosome trisomy was discovered in 4% of the cases (as the sole change); inv(3) and its variations, in 3%; inv(16), in 3%, deletion (9), in3% and 11q23 changes were found in 3% of the samples; t(6;9), in 1%.

In this study we obtained data that are similar with literature data on well established occurrence of genetic AML pathology. However, to determine new diagnostically important aberrations, especially in case of a normal karyotype, use of molecular methods would be required.

PP-31. Fine needle aspiration biopsy (FNAB) of thyroid nodules: one-year review of 10203 specimens

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OBJECTIVES. The aim of the study was to compare FNAB cytological and histopathological results after thyroidectomy.

METHODS. All patients were subjected to FNAB at one institution from January 2015 to December 2015. A total of 1203 specimens were tested (1072 female and 131 male, avarage age 55.8 years). Cytological specimens were stained by Romanowsky-Giemsa-Leischman modification. All cytological results were grouped according to the BSRTC system. Using the Riga East Clinical Hospital information program, we obtained data of 62 histological evaluations for operated patients with cytological findings: atopia of undetermined significance (AUS), follicular neoplasm or suspicion for a follicular neoplasm (FN/SFN), suspicion for malignancy (SFM) and malignancy.

RESULTS. Among the 1203 specimens of FNABs, 11.6% (140) were reported as non diagnostic, 79.7% (959) as benign, 0.6% (7) as AUS, 1.2% (14) as FN/SFN, 3.2% (39) as SFM and 3.7% (44) as malignant. Among 62 histological evaluations, 22.6% (14) were reporded as benign, 61.3% (38) as papillary carcinoma, 8.1% (5) as medullary carcinoma, 4.8% (3) as folliculary carcinoma, 1.6% (1) as anaplastic carcinoma and 1.6% (1) as papillary carcinoma for 1 thyroid lobe and medullary carcinoma for the other. In our study, 39 cases where FNAB results were evaluated as suspicious for malignancy, 18 were operated on, and 10 were determined as malignant. Also, 44 patients in malignant cytological category, 37 were operated on, and 32 were determined as malignant. Among 5 patients whose FNABs were indicated as follicular neoplasms and in whom histological evaluation was performed, surgical pathology findings of 4 cases were reported as malignant. There were no histological data for 9 cases in FN/SFN category.

CONCLUSIONS. We consider that the patients reported as having follicular neoplasms should be operated on. In summary, our study demonstrated that thyroid FNAB, stained by Romanowsky-Giemsa-Leischman modification, is an accurate and relatively precise tool for diagnosis of thyroid malignancy.

PP-32. Latvian experience in surveillance of polio and other enteroviruses circulation

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OBJECTIVES. Laboratory surveillance focusing on revealing enteroviruses in clinical material, especially from patients with acute flaccid paralysis, as well as on virus findings in environmental samples serves as the background for completing the WHO programme and the main control procedure for polio circulation in Latvia.

Detection of the spectrum of circulated non-polioviruses enteroviruses for diagnosis conformation and for surveillance procedures plays the indicator role in the control of polio virus in Latvia.

METHODS. Clinical materials as faeces, CSF, pharyngeal swabs, post mortem collected specimens and sewage, were analysed according to the WHO recommendation. Three different cell lines: L-20B, Hep-2C, RD(A) were used for enterovirus isolation. Isolated polio and enterovirus strains were characterized for serotype on the basis of neutralization reaction with specific antisera. Entero RNS detection in samples was completed using the conventional PCR method.

RESULTS. The last wild polio virus in Latvia was detected in 1965. It was WPV1 isolated from sewage. Since that year only polio vaccine associated viruses have been detected in Latvia. Polioviruses were found in 98 samples of sewage water (Sabin PV1- 38, PV2-29, and PV3-31) and 8 samples of faeces (Sabin PV1-5, PV2-1, and PV3-2).The last polio vaccine virus was detected in 2011. No poliovirus was found in 2012–2015. Active circulation of other enteroviruses was detected in all years.

CONCLUSIONS. Vaccine-associated polio viruses from all polio virus serotypes circulated simultaneously with broad-spectrum non-polio enteroviruses until 2008. During the two-year period, after introducing inactivated polio vaccine in Latvia in 2006, the laboratory detected only sporadic polio vaccine-associated viruses but none in 2012–2015. Obtained data gives us the possibility to characterize the intensity and spectra of polio and non-polio enteroviruses circulating in Latvia, and to estimate and control surveillance of polio virus circulation.

PP-33. Brain-to-brain loop concept in application to porphyrias: 10-year experience at North Estonia Medical Centre

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OBJECTIVES. The concept of "brain-to-brain loop" for laboratory testing was introduced by Lundberg in 1975. According to this concept, the loop begins in the brain of the physician with the postulation of the clinical question and the selection of laboratory tests, followed by sample collection and transportation to the laboratory, as well as by the measurement of analytes at the laboratory and delivery of the result to the physician. The loop closes in the brain of the physician with a decision about patient management.

Applying the brain-to-brain loop concept, the management of porphyrias (P) at our hospital was improved. The aim of our study was to present changes that led to this improvement.

METHODS. We retrospectively analysed changes in the process. Prior to 2011, only alfa-aminolevulinic acid and koproporphyrin were measured in urine by at-home methods. At the beginning of 2011 the P laboratory was merged with the toxicology laboratory and we started with changes in the handling protocol of P, which included standardisation in the preanalytic, analytic and postanalytic steps. The first-line porphyrin tests were broadened by urine porphobilinogen and total porphyrins; additionally, lead analysis was also added for differentiation between lead poisoning and P. Collaboration between laboratory physicians and experienced clinicians was also encouraged to minimise problems caused by communication gaps and to improve the quality of patient management.

RESULTS. When prior to the introduction of the changes, 1612 porphyrins were measured, then after this their number was 1236. Among them, regarding prior to and after changes, 19% and 5% of the results, respectively, were mildly increased and not associated with P, while 1.6% and 5.4% of the results, respectively, were associated with P.

CONCLUSION. The brain-to-brain concept that has been applied in our laboratory for diagnosing of P during recent 10 years has lead to the improvement of the quality of patient-centred medical care in this field.

PP-34. Investigation of porphyrias: 10-year experience at the North Estonia Medical Centre

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OBJECTIVES. Porphyrias (P) are the group of disorders caused by deficiency in one of the enzymes in the heme synthesis pathway. According to clinical presentation, P are usually classified as acute or cutaneous. Acute P is usually clinically present with acute neurovisceral crisis, while cutaneous P is characterized by skin photosensitivity and lesions.

Diagnosis of P can be made by combining clinical information with laboratory testing. When acute P is suspected, it is usually necessary to have three tests for first-line determination: urinary porphobilinogen, alfa-aminolevulinic acid and total porphyrins. In cutaneous presentation, total urinary or plasma porphyrins are more appropriate.

In Estonia, there are only two hospitals where the first-line biochemical methods are available. One of them is North Estonia Medical Centre. Our laboratory receives samples for measurement of porphyrins from all hospitals of Tallinn, capital of Estonia.

We analysed retrospectively porphyrins test utilization, based on the data of the measurements of porphyrins performed at our laboratory in 2006–2015.

METHODS. The data for all porphyrin analyses were drawn from the LIS database.

RESULTS. The laboratory performed 2848 porphyrins analyses for 948 patients in 2006–2015.

Most of the patients with suspicion of P had been referred by physicians of occupational diseases (660), gastroenterologists (159) and neurologists (87), the least of the papients had been referred by dermatologists (13), nephrologists (12), haematologists (6), surgeons and emergency physicians (5), as well as by psychiatrists (4) and cardiologists (2). P was diagnosed either only on the basis of clinical symptoms and porphyrin results (13 patients) or additional DNA analysis (6 patients). All patients with P received appropriate timely treatment.

CONCLUSION. The clinical presentation of P is often non-specific. Our experience in the field of P investigation has demonstrated the importance of the impact of first-line biochemical testing on diagnosis.

PP-35. Application of six sigma for quality assurance at the laboratory of North Estonia Medical Centre

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OBJECTIVES. To comply with the requirements of ISO 15189:2012 we have to constantly assess and monitor the laboratory´s contribution to patient care. Therefore, the quality indicators (QI) of the pre-analytical, analytical and post-analytical phases are measured. One aspect of assuring the quality of laboratory testing is monitoring the level of analytical performance of tests. The aim of this study was to apply the Six Sigma concept for assessment of laboratory performance.

METHODS. The QI and the analytical performance of the test data (28 immunoassay tests, 35 clinical chemistry tests) were registered within a period of one year. The QI chosen for monitoring the pre-analytical (6), analytical (3) and post-analytical (1) phases were measured by calculating defects per million (DPM) and converting the measurement results into the Sigma metric. The analytical performance of the tests was quantified by the Sigma metrics using the equation "Sigma-metric = (TEa% - Bias%)/CV%".

RESULTS. Laboratory performance in the pre-analytical phase ranged between 3.5–5.6 Sigma (σ), in the analytical phase between 3.5–4.5 σ and in the post-analytical phase 5.3 σ. Of the immunoassay tests 71% and of the clinical chemistry tests 54% performed higher than 6 σ. Of the immunoassay tests 4% and of the clinical chemistry tests 20% performed under 3 σ.

CONCLUSIONS. This study shows that the Six Sigma concept is an applicable method for assessing and improving the quality of our laboratory services. The Six-Sigma scale provides a universal benchmark for comparing the quality of our laboratory and its analytical performance of tests with those of other laboratories.

PP-36. Actionable mutations in the whole-genome sequenced gene donors of the Estonian Biobank

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INTRODUCTION. In 2013, the American College of Medical Geneticists (ACMG) issued a minimal list of 24 actionable conditions and 56 related genes that are of medical utility and should be reported back if incidentally found mutated in clinical whole genome and whole exome sequencing.

OBJECTIVES AND METHODS. At the Estonian Genome Center, we have performed whole genome sequencing of over 2200 individuals selected to be a representative subset of the Estonian population with approximately 30x mean coverage. The data is meant to be used for a variety of further population and health-related studies. As one of the aims of our biobank is to provide feedback to the gene donors regarding their health risks, we were searching for the sequencing data for known and expected pathogenic mutations, starting from the ACMG gene list as the first priority.

RESULTS. Based on initial filtering, database and literature search, we were able to detect 33 variants in 20 genes related to 13 clinical conditions in the ACMG list mentioned above. Forty five individuals (2% of the sequenced cohort) carried the variants. One of the surprises was that 16 individuals with known and expected BRCA1 and BRCA2 pathogenic mutations (0.7% of total) were detected in the sequencing cohort.

PERSPECTIVES. The sequencing data will be further validated and used to set up a health-related feedback routine at our biobank, together with required professional counselling.

PP-37. Changes in inflammatory and immunological parameters during progression of renal cell carcinoma

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AIM. To establish inflammatory and immunological markers that significantly correlate with presence, stage and prognosis of RCC.

METHODS. Before treatment, the peripheral blood of patients with suspected RCC was tested for C reactive protein (CRP), erythrocyte sedimentation rate (ESR), lactate dechydrogenase (LDH), white blood cell (WBC), neutrophil (Ne), monocyte (Mo), lymphocyte (Ly) platelet (PLT) count and Ly subpopulations: CD3+, CD3+CD4+, CD3+CD8+, CD38+, CD16+CD56+, CD19+, CD95+ using a flow cytometer. The study was launched on 25.11.2013 with follow up of 158 patients from a single institution until 01.12.15.

RESULTS. Nineteen patients had benign tumours, 139 had RCC, among them 85 with stage I, 9 with stage II, 12with stage III and 33 with stage IV or with recurrence. Between the groups of local and advanced RCC stages there was found a statistically significant difference for: WBC ($p = 0.02$), Ne ($p = 0.05$), PLT ($p = 0.001$), Mo ($p = 0.01$), CRP ($p = 0.001$), LDH ($p = 0.008$), PLT/Ly ($p = 0.001$), Mo/Ly ($p = 0.019$). The only variable that apparently differed between the patients with benign and malignant tumours was Mo/Ly ratio, although this difference did not reach statistical significance ($p = 0.057$). In 45 patients with the advanced disease, a statistically significant difference in survival distributions between the groups with lab results above or below median values was observed for PLT ($p = 0.002$), CRP ($p = 0.01$), ESR ($p = 0.002$), CD8+/CD19+ ($p = 0.023$), PLT/Ly ($p = 0.049$).

CONCLUSIONS. The most significant correlation with stage of RCC was proved for PLT, Mo, CRP, ESR, CD19+ and PLT/ Ly ratio. A difference in Mo/Ly ratio was observed in association with presence of malignancy, which might serve as a useful diagnostic marker. The PLT, CRP, ESR, PLT/Lyand CD8+/CD19+ may predict survival in advanced RCC.

PP-38. Increase of immature granulocytes in septic patients within the first 24 hours of hospitalisation

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BACKGROUND. Studies have shown that the count of immature granulocytes (IG) increases in the early stages of sepsis. We analysed how many patients who are diagnosed with sepsis at hospitalisation have abnormal level of IG, and whether this is related to procalcitonin (PCT) values, causative bacteria and disease outcome.

MATERIAL AND METHODS. The study is based on the medical records of patients from the Internal Medicine Clinic of Tartu University Hospital. The results of 46 patients aged 27–91 (mean 70years) were analysed, among them 28 with the diagnosis of sepsis and 18 without this diagnosis but with infection. In the first group 14/28 were men, in the second; 7/18. Six patients died during 30 days after hospitalisation and one patient died 2 months later: thus there were 7 deaths. We compared the IG and PCT values measured within the first 24 hours. The IG was assayed with Sysmex XE-2100 or XE-5000 hematological analyser, using flow cytometry; PCT was assayed by the ECLIA method. An IG count over 0.06 x 10^9/l, IG% over 0.6%, and PCT over 2 ng/ml were considered increased. In the septic group, blood cultures were gram-negative (G-) in 16 cases and gram-positive (G+) in 6 cases; 2 G-/G+ bacteria were isolated, in 4 cases the pathogen was not isolated.

RESULTS. The IG count was elevated in 13/28 patients in the sepsis group and in 4/18 in the infection group; IG% was elevated in 10/28 and 2/18, and PCT was elevated, in 16/28 and 9/18, respectively. The CRP was 4–416 mg/l, < 10mg/l occurred in only 2 cases; the mean value being 160 mg/l. There was no correlation between IG and PCT. From the blood cultures of patients with an increase in IG, 7 G- and 4 G+ pathogens were isolated, bacteria were not cultivated in 2 cases. The patients who died had shown abnormal IG count in 5/7, IG% 3/7 and PCT in 4/7 during the first 24 hours. Most of them (5/7) had G- bacteria.

CONCLUSIONS.

1. An increase in IG and PTC was found both in septic and non- septic patients during the first 24 hours of hospitalisation.
2. An increase in IG was found in patients with both G+ and G- infections.
3. The IG is a well available test which could provide useful diagnostic and prognostic information.
4. This pilot project should be carried on to make more detailed conclusions.

Alphabetical Index of Authors

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