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# Laboratorinė MEDICINA

## Program and Scientific Abstracts Book



**BALM** Baltic Association of Laboratory Medicine



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Latvijas  
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## WELCOME SPEECH OF THE CONGRESS PRESIDENT

*It is with great pleasure and excitement that I welcome you to the XVII Baltic Congress of Laboratory Medicine here in the beautiful and historic city of Vilnius.*

*As we gather in this vibrant city, we are united by our shared commitment to advancing the field of laboratory medicine. This highly anticipated event promises to be an enriching and enlightening experience for all of us. Over the next few days, we will have the opportunity to engage with leading experts, researchers, and professionals who have traveled from various corners of the world to share their knowledge and insights.*

*Our Congress is designed to foster the exchange of ideas, showcase the latest advancements, and highlight best practices in laboratory medicine. We have an outstanding program lined up, featuring a diverse range of presentations, workshops, and discussions that will undoubtedly inspire and challenge us to push the boundaries of our field.*

*I encourage you to take full advantage of this unique platform to network, collaborate, and learn from one another. Together, we can drive innovation and improve patient care, making a significant impact on the health and well-being of our communities.*

*Thank you for your dedication to the field of laboratory medicine and for your participation in this Congress. I look forward to the fruitful discussions and new friendships that will emerge from our time together.*

Assoc. prof. Dalius Vitkus, PhD, EuSpLM  
Congress President

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## General Information

### Vilnius:

Vilnius, the capital of Lithuania, is a city rich in history, culture, and charm. Known for its stunning architecture, Vilnius boasts one of the largest and most beautiful old towns in Eastern Europe, a UNESCO World Heritage site. The city offers a mix of Gothic, Renaissance, Baroque, and Neoclassical buildings, making it a picturesque destination. Vilnius is also known for its green spaces, such as Bernardine Park and Vingis Park, providing a perfect blend of urban and natural beauty.

### LITEXPO:

LITEXPO, the Lithuanian Exhibition and Congress Center, is the largest conference and exhibition venue in the Baltic States. Located just a short drive from the city center, LITEXPO offers state-of-the-art facilities, including spacious exhibition halls, versatile conference rooms, and advanced technical equipment. It hosts a wide range of international and local events, such as trade shows, conferences, and cultural exhibitions, making it a hub for business and networking opportunities in the region. With its excellent services and infrastructure, LITEXPO is the ideal location for the XVII Baltic Congress of Laboratory Medicine.

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## Assessment of Stability of Activated Partial Thromboplastin Time, D-Dimer, Fibrinogen, and Thrombin Time Under Different Storage Conditions in Human Plasma

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**Keywords:** Coagulation, storage time, storage temperature, stability.

**Introduction.** In this study, we aimed to determine the effects of storage time and temperature on commonly performed coagulation tests such as activated partial thromboplastin time (APTT), D-dimer (DD), fibrinogen (FBG), and thrombin time (TT) in human plasma.

**Materials and methods.** Whole blood samples from 80 patients were collected in a 3.2% sodium citrate vacutainer. The blood was centrifuged within two hours of collection at an RCF of 2,000 g for 15 minutes, and the platelet-poor plasma obtained was analyzed for APTT, DD, FBG, and TT tests. For APTT, DD, and FBG tests, the remaining sample was split into two parts. The first part was kept at room temperature, testing was repeated at 4 hours, 6 hours, and 8 hours from sample collection time. The second part was frozen at -18°C, testing was repeated the following day, 24 hours from sample collection time. For TT tests, the sample was only frozen and tested the following day, 24 hours from sample collection time. Tests were performed on a fully automated coagulation analyzer. The percentage change of the results from baseline (first test) for APTT, DD, FBG, and TT tests was also studied.

**Results.** A percentage change of more than  $\pm 5\%$  from baseline was considered as a clinically significant change. A total of 79 samples were evaluated. In the freezer, the samples were stable for DD, FBG, and TT tests at 24 hours, showing a change of  $<5\%$  from baseline. In comparison, the samples for the APTT test were not stable at 24 hours, showing a change of 10.6% on average. At room temperature, the samples were stable for DD and FBG tests for up to 8 hours, and for APTT tests for up to 4 hours, showing a change of  $<5\%$  from baseline. However, the samples for the APTT tests were not stable at 6 hours and 8 hours, showing a change of 9.0% and 9.3% on average, respectively, from the baseline.

**In conclusion,** the patient plasma samples for DD, FBG, and TT tests could be safely stored for up to 24 hours in the freezer, whereas the samples for APTT could not. At room temperature, samples for DD and FBG tests could be safely stored for up to 8 hours, while the samples for APTT could be stored for up to 4 hours.

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## Changes in Fatty Acid Composition of Extracellular Vesicles Obtained from Patients with a History of Myocardial Infarction

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**Keywords:** myocardial infarction, extracellular vesicles, fatty acid composition, malondialdehyde, infrared spectroscopy.

**Introduction.** Myocardial infarction and ischaemia cause around 16% of deaths and is the main cause of death worldwide. Atherosclerosis-induced coronary heart disease is a major risk factor for myocardial infarction. Extracellular vesicles have been associated with numerous pathophysiological processes in atherosclerosis and they are emerging as a novel biomarker in diagnostics.

**Aims and Objectives.** This study aims to identify significant differences in exosome fatty acid composition between healthy individuals and patients who have experienced myocardial infarction and evaluate the correlation between individual fatty acids and malondialdehyde.

**Material and Methods.** The study includes healthy men aged 25-60 and men aged 40-60 who have experienced myocardial infarction. Fatty acid composition of extracellular vesicles was analysed using gas chromatography mass spectrometry (GCMS), and molecular vibration spectra were recorded using Fourier-transform infrared spectroscopy (FTIR).

**Results.** Results showed statistically significant differences in volume of saturated fatty acids C14: 0 and C16: 0, and monounsaturated fatty acids C18: 1 $\omega$ 9 and C20: 1 $\omega$ 9 between study groups. Significant correlations of malondialdehyde concentration with C14: 0, C18: 3 $\omega$ 3, total  $\omega$ 3 fatty acids, and the ratio of C18: 2 $\omega$ 6 to C20: 4 $\omega$ 6 fatty acids were observed. FTIR analysis revealed differences in lipid bands at 2960 cm<sup>-1</sup>, 2852 cm<sup>-1</sup>, 1740 cm<sup>-1</sup>, and 1453 cm<sup>-1</sup>, and protein bands at 1650 cm<sup>-1</sup> and 1545 cm<sup>-1</sup> between study groups. High sensitivity and specificity classification models were developed using both fatty acid composition and FTIR spectra. Results suggest that changes in extracellular vesicle fatty acid composition may serve as potential biomarkers for myocardial infarction. This could contribute to a better understanding of the pathological processes of myocardial infarction and the development of more accurate and effective diagnostic strategies. The study emphasizes the need for further standardization of extracellular vesicle isolation and analysis methodologies to ensure data reliability and reproducibility in future research.

**Conclusions.** In summary, this work provides significant evidence that extracellular vesicle fatty acid compositions and their molecular vibration spectra can differentiate MI patients from healthy individuals. Future studies should combine fatty acid composition and FTIR spectra in creating a comprehensive classification model.

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### Genetic insights into cystic kidney diseases: phenocopy detection through next-generation sequencing

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**Keywords:** cystic kidney disease, genetic testing, phenocopies.

**Introduction.** Cystic kidney diseases represent a group of genetically heterogeneous disorders with pathogenic variants in PKD1 and PKD2 genes being the most common (about 94%) cause of autosomal dominant polycystic kidney disease (ADPKD). While clinical diagnosis can often be easily made upon clinical imaging criteria, the implications of next-generation sequencing not only allow to investigate the structurally complex PKD1 gene and confirm a diagnosis, but also unveiled the existence of various phenocopies. Aim. To identify and classify genetic variations within kidney-associated genes among patients presenting with multiple renal cysts, with a particular focus on detecting non-PKD1/2 genetic causes.

**Materials and Methods.** 76 patients with multiple renal cysts were included in the study. The study group consisted of 43 females and 33 males: 69 adults and 7 children. Patients were tested with a kidney-focused next-generation sequencing panel of 498 genes. The pathogenicity of detected variants was assessed using the American College of Medical Genetics and Genomics guidelines. Pathogenic/likely pathogenic variants and variants of uncertain significance (VUS) were reported.

**Results.** Phenotype-related variants were identified in 61 patients (80.2%). The most frequently involved genes were PKD1 (40 patients, 65.6%) and PKD2 (11 patients, 18%). In PKD1 gene 32 variants were classified as pathogenic/likely pathogenic, and 8 as VUS. 22 variants were not described in the literature. The remaining variants were detected in non-PKD1/2 genes: COL4A5 (2 patients, 3.3%), PAX2 (2 patients, 3.3%), COL4A1 (1 patient, 1.6%), PKHD1 (1 patient, 1.6%), NPHP1 (1 patient, 1.6%), GANAB (1 patient, 1.6%), HNF1B (1 patient, 1.6%), and UMOD (1 patient, 1.6%). In these cases, two patients were clinically diagnosed with ADPKD, so the existence of phenocopies was observed in our study.

**Conclusions.** The high variant detection rate among patients with cystic kidney disease reflects results similar to those of other studies worldwide, and a genetic diagnosis was established for the majority of patients in our study. Previously undescribed novel variants were identified. As expected, some non-PKD1/2 patients demonstrated phenotypes resembling or identical to classic ADPKD. For patients, genetic confirmation is the basis of personalised patient care and bears significant midterm to long-term prognostic value.

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### Spread of parvovirus B19 in Latvia in winter-spring of 2024

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**Keywords:** Parvovirus B19, infection, outbreak.

**Background.** Outbreaks of parvovirus B19 (B19V) infection are observed periodically, every four to ten years. People tend to get infected with B19V more frequently at the end of winter, in spring, and at the beginning of summer. The last B19V infection outbreak in Latvia was in 2018 (around 30% positive). From 2019 to 2023, 4% of samples tested were positive for B19V, whereas in 2024, this percentage rose to 52% of samples. Detailed epidemiological analysis is lacking because B19V is not under strict surveillance in most countries. Available data indicates that in the spring of 2024, several countries experienced an increase in infection rates.

**Objectives.** This study provides an analysis of the B19V outbreak in Latvia during the first quarter of 2024.

**Material and Methods.** During the B19V outbreak in Latvia from January 1 to March 31, 2024, clinical samples from 457 patients (children 59%, women 36%, and men 5%) were tested at the National Microbiology Reference Laboratory (NMRL). Among these, 45 patients were tested using both PCR and ELISA (IgM and IgG), 5 patients with PCR and ELISA (IgM), 1 patient with PCR and ELISA (IgG), 88 patients only with PCR, 169 patients with ELISA (IgM and IgG), 117 patients only with ELISA (IgM), and 32 patients only with ELISA (IgG). Urine, saliva and blood samples were tested for B19V DNA using real-time PCR. Blood samples were tested using the ELISA method to detect B19V IgM and IgG antibodies.

**Results.** B19V infection was laboratory confirmed by real-time PCR (positive in 70/139 - 50%), ELISA IgM (positive in 163/336 - 49%), and ELISA IgG (positive in 175/247 - 71%). Overall, 52% (222/425) of patients tested by PCR and/or ELISA IgM were laboratory confirmed positive for B19V. Among children, B19V was positive in 36% (153/425) and negative in 26% (110/425) when tested by PCR and/or IgM. Among women, 14% (60/425) tested positive and 19% (79/425) tested negative. Among men, 2% (9/425) tested positive and 3% (14/425) tested negative. IgG antibodies were not tested in 71 out of 457 patients, which is 16% of the total.

**Conclusions.** At the beginning of 2024, 92% of positive cases were observed in children and women aged 29 to 42 years. Considering these circumstances, the implementation of screening measures should be considered, especially for high-risk groups such as pregnant women. Laboratory confirmation would be crucial to differentiate B19V infection from other infectious diseases that also present with rashes (measles, rubella, HHV6, etc.).

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## Molecular identification of acute viral gastrointestinal infection causative agent

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**Keywords:** Molecular, gastrointestinal infection, diarrhea, Rotavirus, Norovirus.

**Objectives.** Acute gastrointestinal (GI) infection can be caused by various infectious agents such as virus, bacteria or parasites, distinguish the ethiological agent of diarrhea is essential. GI viruses can cause acute diarrhea in infants below the age of 5, what could be fatal. Identifying virus types is crucial for monitoring regional outbreaks. In the period 01.2019 – 03.2024 in Latvia were registered 11 100 viral GI infection cases - 3730 (34%) of them were caused by Rotavirus, accordingly to data of the Centre for Disease Prevention and Control (CDPC).

**Materials and methods.** In the period 01.2019 – 03.2024 overall 526 faecal samples from different regions of Latvia were tested by real time PCR (Allplex GI-Virus Assay, Seegen). Nucleic acid was extracted by NucliSENS easyMAG (BioMeriux) automated system. The patient age ranged from infant to 97 years with male/female ratio 243/283.

**Results.** We obtained 100/526 (19%) GI virus positive cases. Positive GI virus proportion were: Norovirus G2 (NVG2) - 46/100 (46%), Rotavirus (ROV) - 23/100 (23%), Norovirus G1 (NVG1) and Sapovirus (SAV) - 9/100 (9%), Astrovirus (ASV) - 3/100 (3%), Adenovirus (ADV) - 2/100 (2%), NVG2 and SAV combination - 4/100 (4%), NVG1 and NVG2 combination - 3/100 (3%), NVG2 and ASV combination - 1/100 (1%). The infected male/female ratio was 48/52 with average age 38.7, that included 29 (29%) kids. The most part of infected children 24/29 (83%) were below the age of 5.

**Conclusions.** According molecular test results the most represented were NVG2 (46%), half less common - Rov (23%), SAV and NVG1 distribution were the same (9%), much less common were other viruses as ASV and ADV, combined GI virus infections were observed rarely as well. Although according to our data the most distributed were NVG2, epidemiological data from CDPC showed the higher ROV infection rate (34%) in Latvia, unfortunately was not available epidemiological data about NVG2 and other GI virus infection rate, supposedly epidemiologically prevailed NVG2 as well and ROV rate was lower because of the successful immunization program of children in Latvia. Almost third part of tested positive samples were childrens faecal samples 29/100 (29%), although only 50/526 (9%) from tested samples belonged to children. Most of GI virus infected children (83%) belonged to risk group (<5 years).

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## Incidence and Primary Immunophenotypic Characterization of Acute Lymphoblastic Leukemia (ALL) by Flow Cytometry at the Children's Clinical University Hospital from 2022 to 2024

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**Keywords:** Acute lymphoblastic leukemia (ALL), B-cell ALL (B-ALL), T-cell ALL (T-ALL), ALOT, NOPHO.

**Introduction.** Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in pediatric patients, accounting for more than 25% of all pediatric cancer cases. Although the etiology of ALL can be attributed to inherited genetic syndromes such as Down syndrome and Fanconi anemia, as well as congenital immunodeficiency disorders including Wiskott-Aldrich syndrome, in some patients, the underlying cause remains largely unidentified in the majority of cases.

**Aims and Objectives.** To investigate the incidence and primary immunophenotypic characterization of pediatric ALL cases using flow cytometry at the Children's Clinical University Hospital from August 24, 2022, to January 31, 2024.

**Materials and Methods.** This retrospective cohort study included 26 pediatric oncohematological patients diagnosed with C91.0 (ALL according to the ICD-10). The study evaluated patient demographics including age, gender, and region of residence. Patients underwent an acute leukemia orientation tube (ALOT) protocol examination, as per the standardized NOPHO protocol, at the Clinical Laboratory of the Children's Clinical University Hospital. Additionally, further B- or T-lymphoblast leukemia phenotyping was conducted to ascertain the primary immunophenotype of the blasts.

**Results.** The incidence of ALL determined by flow cytometry at the Children's Clinical University Hospital from August 24, 2022, to January 31, 2024, revealed a total of 26 patient cases. Of these, 23 cases (-88%) were diagnosed with B-cell ALL (B-ALL), and 3 cases (-12%) with T-cell ALL (T-ALL). 16 out of 26 patients (-62%) resided in Riga/Riga district. Among the 26 patients, 16 were female (-62%) and 10 were male (-38%). At the time of ALL diagnosis, the most common age was 2-3 years old (8 out of 26 cases, corresponding to 31%). The most common positive markers identified in primary immunophenotyping for B-ALL were CD10, CD19, CD22, cyCD22, CD24, CD34, CD38, CD58, CD73, cyCD79a, CD81, and CD123, but in cases of T-ALL, the markers included CD2, cyCD3, CD5, CD7, CD99, and nTdT.

**Conclusions.** This study demonstrated that out of 26 patient cases, B-ALL was most frequently detected, occurring predominantly in the age group of 2-3 years among females residing in Riga/Riga district. To investigate the incidence and primary immunophenotypic characterization of pediatric ALL cases more precisely, this retrospective cohort study should be extended over a longer period of time.

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## Results of the first pilot run of *Salmonella* spp WGS in Lithuanian public health surveillance laboratory

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**Keywords:** *Salmonella*, WGS, outbreak investigation.

**Introduction.** Phenotypic serotyping has been used in suspected outbreaks of *Salmonella* spp. Implementation and development of sequencing research and its use under European funding.

**Aims and Objectives.** The aim of the research was to suspect possible outbreaks in different geographical locations and different time periods after performing the sequence count. *Salmonella* strains were selected by epidemiologists from sented to NPHSL for serotyping.

**Materials and Methods.** Archived cultures have been recovered using the following mediums XLD agar and HEA agar. DNA extraction was performed using the EZ1&2 Virus Mini Kit v 2.0 (Qiagen). Sequencing library preparation was performed with the Nextera XT Library Prep Kit (Illumina). Sequencing was performed using a MiSeq Dx sequencer using Miseq Reagent Kit v2 500 cycles (Illumina). Sequencing results were evaluated by the SeqSphere program and using *S. enterica* cgMLST v2 scheme from Enterobase (3002 loci).

**Results.** Clusters were determined with core-genome multi-locus sequence typing. Isolates with < 5 allelic differences (AD) were considered to belong to the same cluster. Were determined 5 clusters. The biggest one was *Salmonella enterica* *Enteritidis* (ST 11, cgMLST 468). Cluster included 14 isolates from 2 cities. The AD between different isolates was 0.

**Conclusions.** After the implementation of the NGS method, an outbreak was identified and confirmed for the first time in different geographical locations, when it was only possible to suspect it using serotyping methods. Sequencing results can be compared with data of State Food and Veterinary Service to assess potential sources of contamination. The resulting data can be used to publish on the EpiPulse platform and to search for the cross-border spread of bacteria. NGS brings significant knowledges to FWD outbreak investigation and would be routinely performed.

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## Research in the field of validity of the new anti-malarial coartem drugs version

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**Keywords:** new anti-malaria coartem, tropical malaria.

**Introduction.** The role of anti-malaria therapy in managing acute tropical malaria, in children, is quite controversial, with occasionally varying results when either, administered alone or with Paracetamol BP. The purpose of this study was to assess the efficacy of the standard six (6) doses regimen of Coartem (Artemether Lumefantrine) in the treatment of children diagnosed with non-complicated falciparum malarial infection.

**Material and Methods.** This was an open-label study, wherein, children 1-10 years of age, weighing from 5-25 Kg(s) and with a clinical presentation compatible with the infection were eligible for participation. A group of 360 total participants enrolled, out of whom, 180 children, with uncomplicated infections, were subjected to the standard six (6) doses of Coartem (Artemether 120 mg plus Lumefantrine 20 mg), with no Paracetamol, over three (3) days, concurrently with the administration of the similar Coartem dosage along with Paracetamol Bp 500 mg to the remaining 180 patients, over the same period, in addition to which a systematic follow up of 1 week, 2 weeks and 4 weeks, was effected.

**Results.** In both groups, the treatment seemed to fairly, rapidly clear parasitemia and fever, in that, in those with Coartem administered with Paracetamol, the overall cure rate was 84.9% after the 4 weeks, in contrast with 75.5% in those with no Paracetamol, further to which both rates were over 4% higher when corrected by PCR for re-infection. Cure rates at 1 week and 2 weeks were quite similar and high in both groups, reaching up to 97% and 88%, for those with a combination of Coartem and Paracetamol and for those with Coartem alone, respectively, further to which higher corresponding rates of 99% and 90% respectively, after PCR correction arose. Adverse eventualities were mostly mild and with no cardiotoxicity, as evidenced by electrocardiographic evidence, and hence, the treatment appeared to be generally safe and well tolerated in both groups.

**Conclusions.** Tropical malaria, which is a multi-drug resistant plasmodium falciparum infection, has its main current standard prescription as the Co-artemether six (6) doses regimen, however, its co-administration along with Paracetamol Bp 500 mg, as a first line pain killer, tends to result into not only significantly rapid, but surprisingly more cures than when administered alone.

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## Confirmatory testing for the carbapenem-non-susceptible and carbapenemase producing Enterobacterales by whole genome sequencing and rapid PCR assay in the National Microbiology Reference Laboratory of Latvia

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**Keywords:** Enterobacterales, carbapenemase, whole genome sequencing WGS.

**Introduction.** Referral of the carbapenem-non-susceptible and/or carbapenemases producing clinical isolates of Enterobacterales to the National Microbiology Reference Laboratory (NMRL) for confirmatory testing is mandatory in Latvia. Recently, the confirmatory PCR assay was complemented by the whole genome sequencing (WGS).

**Aims and Objectives.** We evaluated the results of the rapid PCR assay with WGS as a control.

**Material and Methods .** 397 Enterobacterales isolates (26 *Citrobacter* spp., 8 *Enterobacter* spp., 33 *Escherichia* spp., 325 *Klebsiella* spp., 1 *Morganella* spp., 2 *Proteus* spp., 1 *Raoultella* spp., 1 *Serratia* spp.) received from central and regional hospitals (Jul-2022 to Feb-2024) were assessed by WGS and Xpert Carba-R (Cepheid, USA). Libraries prepared with DNAPrep (Illumina, USA) and sequenced either on Illumina NextSeq550 or NovaSeq6000 in 150PE or 250PE configuration (ave. 5 million paired-end reads per sample). Bioinformatic analysis performed by an in-house pipeline Ardetype v0.1.0-dev (Bodrenko & Vangravs, Github), employing Fastp v0.22.0, Shovill v1.1.0, AMRFinderPlus v3.10.42 (database v2022-10-11.2), ResFinder v4.1.11 (database v2023-03-29), RGI v5.2.1 (card\_v3.1.4), 7-gene MLST v2.19.0 (PubMLST schemes), and Kleborate v2.2.0. At least two votes from three AMR gene databases were considered for the presence of a gene.

**Results.** WGS enabled differentiated detection of blaOXA-244 in *E. coli* (2/3 ST10, 2/3 ST167), blaNDM-5 (2/3 *E. coli* ST167, 4/4 ST361), blaNDM-2 (1/119 *K. pneumoniae* ST147), blaNDM-1 (6/9 *C. freundii* ST540, 1/2 *K. pneumoniae* ST45, 70/119 ST147, 1/1 ST147-1LV, 1/1 ST147-3LV, 1 *P. mirabilis*, 1 *R. ornithinolytica*), blaKPC-2 (1/5 *K. pneumoniae* ST37, 2/2 ST39, 3/9 ST307, 1/1 ST392, 10/10 ST395-1LV), blaKPC-3 (1/1 *E. coli* ST73, 39/40 *K. pneumoniae* ST512, 1/5 ST5020, 1 *M. morgani*), and blaVIM-1 (1/4 *C. freundii* ST8). Beside these double tested isolates, the systematic genomic surveillance has enabled NMRL to spot the NDM-5 carbapenemase also in *K. pneumoniae* ST395, for the first time in Latvia, isolated Oct-2023.

**Conclusions.** Since the whole genome sequencing provides fine detection of the full carbapenemase spectrum in Enterobacterales combined with the genomic surveillance, the results question the need for the PCR assay in the era of whole genome sequencing.

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## Clinical Laboratory Experience in Fetal Aneuploidy Detection: A Comparative Analysis of NGS-based and dPCR-based NIPT Methods

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**Keywords:** NIPT, dPCR, NGS.

**Introduction.** Non-invasive prenatal testing (NIPT) utilizes cell-free fetal DNA (cfDNA) from maternal plasma to detect fetal aneuploidies. While next-generation sequencing (NGS) and microarray-based NIPT are established methods, digital PCR (dPCR) has emerged as a promising alternative.

**Aims and Objectives.** This study compares the performance of NGS and dPCR in detecting fetal aneuploidies involving chromosomes 13, 18, 21, and X. We evaluate sensitivity, specificity, and efficiency of both methods and assess dPCR's feasibility as a cost-effective alternative to NGS-based NIPT.

**Material and Methods.** We analyzed 110 samples with an average gestational age of 14 weeks ( $\pm 4.4$  SD) using NGS and dPCR methods for NIPT.

**Results.** Comparative analysis showed no significant difference in sex determination between the two methods. dPCR-based NIPT estimated a reduced fetal fraction by 3.7 percentage points (29% for males, 34% for females,  $p < 0.001$ ). Specificity for dPCR NIPT (chromosomes 21, 18, 13, X) was 98.1%, 94.8%, 100%, and 92.2%, respectively, with a sensitivity of 100%. NGS-based NIPT demonstrated higher specificity (>99%). Case Report: In a detailed case study involving a confirmed vanishing twin, NGS-based NIPT indicated no aneuploidy and suggested a male fetus. Conversely, dPCR NIPT detected a fetal fraction of 6.2%, with a Y chromosome count of 1.8%.

**Conclusion.** dPCR-based NIPT offers rapid (~24-hour turnaround) and cost-effective fetal aneuploidy screening compared to NGS-based NIPT. Integration of both methods holds promise for enhancing clinical accuracy and efficiency in prenatal screening.

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## Detection of Azole-Resistant *Aspergillus fumigatus* by Polymerase Chain Reaction

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**Keywords:** aspergillosis, *aspergillus fumigatus*, azole resistance.

**Introduction.** Aspergillosis is an infectious disease caused by inhaling *Aspergillus* mold spores from the environment. *Aspergillus fumigatus* is the most common causative agent of aspergillosis. In 2023, at the REUH laboratory, 63 *Aspergillus* spp. were isolated. Among these, *A. fumigatus* accounted for 87.3%, with other common non-*fumigatus* *Aspergillus* species including *A. flavus* (9.5%), *A. niger* (3.1%), and no *A. terreus*. First-line therapy for aspergillosis is voriconazole, a triazole antifungal, along with other azoles. However, azole resistance is a growing global health issue, with rates ranging from 0% to 19% worldwide. The main resistance mechanisms involve point mutations in the *cyp51A* gene, along with a 34-base pair (TR34/L98H) and a 46-base pair (TR46/Y121F/T289A) tandem repeat in its promoter region. The *cyp51A* gene is found in both wild-type and mutant strains.

**Aims and Objectives.** This study aimed to analyze the prevalence of azole-resistant *Aspergillus fumigatus* and to improve diagnostics in Latvia.

**Materials and Methods.** From 01.12.2023 to 01.04.2024, 16 samples from 14 patients were tested at the NMRL of Latvia to detect *A. fumigatus* *cyp51A* gene, TR34, and TR46 mutations using the AsperGenius 2.0 Resistance TR Multiplex real-time PCR kit (PathoNostics, Netherlands). The sample materials were fungal culture (n = 10), bronchoalveolar lavage (BAL) (n = 5), and sputum (n = 1).

**Results.** The analysis of data showed that the *A. fumigatus* *cyp51A* gene was found in 14 samples (from 12 patients) and was not found in two BAL samples (from 2 patients). TR34 and TR46 mutations were not detected in any of the 16 samples (from 14 patients). The male-to-female ratio was 8:6. The ages ranged from 1 month to 97 years, with a median age of 55 years. Of the total, 14.3% (2 out of 14) were children (1 month to 17 years) and 85.7% (12 out of 14) were adults (18 to 80 years).

**Conclusions.** This test was applied for the first time in Latvia and found no TR34/TR46-resistant strains. The PCR assay will help timely detect azole-resistant strains, initiate alternative therapy, and control the prevalence in Latvia. More samples are needed to thoroughly assess the prevalence in Latvia. Direct resistance detection via PCR simplifies and accelerates obtaining results.

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## SARS-CoV-2 surveillance in wastewater in 2023-2024 during the season of acute respiratory infections in Lithuania

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**Keywords:** SARS-CoV-2, wastewater, public health.

**Introduction.** Wastewater studies are increasingly becoming an essential component of public health surveillance, as patients with mild forms of certain diseases do not seek medical attention, thus the epidemiological situation is not accurately assessed concerning these diseases. According to European recommendations, in Lithuania wastewater surveillance was started in Vilnius, Kaunas, and Klaipėda - cities with populations exceeding 100,000 residents.

**Aims and Objectives.** The aim of the studies was to assess the presence of pathogens in wastewater in comparison with the registration of clinical case incidences. The objective was to determine the significance of such studies in evaluating the epidemiological situation and the potential actions for public health institutions.

**Materials and Methods.** Wastewater samples were collected using a 24-hour method. Samples were taken from the wastewater treatment plants in Vilnius, Kaunas, and Klaipėda weekly on the same days. The wastewater analysis was conducted using a concentration method on magnetic particles with the "KingFisher Flex" (ThermoFisher Scientific, USA) instrument. Amplification was performed using a digital PCR instrument "QIAcuity One" (QIAGEN, Germany).

**Results.** The quantities of virus copies obtained from the digital PCR were recalculated considering the population of the serviced area and the daily wastewater flow rate. On average, virus copy numbers were detected with fluctuations. During the influenza and acute respiratory infection season the average virus copy numbers were  $13,212 \pm 2,0116$  (242 to 109,490) copies, during the off-season, an average of  $2232 \pm 1952$  (110 to 6981) copies and in the summer, an average of  $1589 \pm 1501$  copies.

**Conclusions.** The lot of big fluctuations SARS-CoV-2 in wastewater show possibility evaluate illness for public health in different geographical regions and seasons. It is necessary to explore the possibility of detecting other infectious disease pathogens in wastewater and to evaluate their significance from a public health perspective.

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## Analysis of Legionella spp. from water samples in Estonia

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**Keywords:** water, *Legionella*, public health, Estonia.

**Introduction.** *Legionella* is a genus of bacteria that can cause a pneumonia-like illness called Legionnaires' disease. It is common in water environments and can propagate in incorrectly maintained water systems, pools or cooling towers. It can cause infection upon inhalation, which makes pools, showers and cooling towers the main sources of *Legionella*.

**Aims and objectives.** The aim of this study was to analyse the results of the conducted *Legionella* water sample analyses. The objectives were to determine the amount of positive samples, their levels and their species distribution.

**Materials and methods.** All water samples were analysed in the Republic of Estonia Health Board Laboratory of Communicable diseases according to the standard EVS-EN ISO 11731:2017 using the membrane filtration method with washing procedure. The results were analysed using Microsoft Excel.

**Results.** In 2023 755 water samples were analysed, of which 340 (45%) tested positive for *Legionella* with 170 samples having a *Legionella* level of over 1000 CFU/1000 ml (23%). Most of those were *Legionella pneumophila* (244) and 48 of those were *Legionella pneumophila* serogroup 1. In 68 samples another *Legionella* species was detected and in 28 samples *Legionella pneumophila* and another *Legionella* species were detected at the same time. The most common *Legionella* species detected other than *Legionella pneumophila* was *Legionella anisa*, in 35 samples. Other *Legionella* species detected were: *Legionella cherrii*, *Legionella erythra*, *Legionella rubrilucens*, *Legionella bozemanæ* and *Legionella geestiana*.

**Conclusions.** In 2023 in Estonia *Legionella* was detected in almost half of the analysed water samples (45%) with almost a quarter of them (23%) having a level higher than the limit in the drinking water regulation (1000 CFU/1000 ml). 6% of those were *Legionella pneumophila* serogroup 1 which is the primary cause of Legionnaires' disease. However in 13% of the samples another *Legionella* species than *Legionella pneumophila* was detected which shows the importance of being able to also detect *Legionella* species other than *Legionella pneumophila*.

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## Prevalence of cultivated dermatophytes and yeasts in Latvia

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**Keywords:** Fungal infections, dermatophyte, *Trichophyton rubrum*, *Candida* spp..

**Introduction.** Fungal infections usually affect skin, hair, nails or mucous membranes but they can also infect lungs or other parts of body. There are two major causes of fungal infections of the skin and nails: dermatophytes and yeasts (*Candida* spp.). Dermatophytes consist of three genera *Trichophyton*, *Microsporum*, and *Epidermophyton*. *Candida* spp. causes skin and mucous membrane infections called candidiasis.

**Aims and Objectives.** The aim of study was to analyze the most common dermatophytes in 2023 in Latvia.

**Materials and Methods.** 2584 clinical samples were obtained from nails (n=1484), skin (n=871) and hair (n=229) in 2023. Traditional inoculation methods are fungal culture. The fungal species can be identified by observing the distinct morphological colonies on the culture media, examining the characteristics of conidia under a light microscope and culture identification by MALDI-ToF.

**Results.** We obtained 2054/2584 (82.6%) negative dermatophyte results and 450/2584 (17.4%) positive dermatophyte, 184/2584 (7.1%) positive *Candida* spp. Isolated on culture *Trichophyton* 429/450 (95.3%): *T. rubrum* 369/450 (82.0%), *T. mentagrophyte* 29/450 (6.4%), *T. tonsurans* 20/450 (4.4%), *T. violaceum* 1/450 (0.2%), *T.schoebinii* 1/450 (0.2%), *T.interdigitale* 1/450 (0.2%), *Trichophyton* spp. 9/450 (2.0%); *Mirosporum* 20/450 (4.4%): *M.canis* 17/450 (3.8%), *M.gypseum* 1/450 (0.2%), *M.persicolor* 1/450 (0.2%), *Mirosporum* spp. 1/450 (0.2%); *Epidermophyton floccosum* 1/450 (0.2%). *Candida* 184/2584 (7.1%): *C.parapsilosis* 78/184 (42.4%), *C.albicans* 40/184 (21.7%), *C. guilliermondii* 18/184 (9.8%), *C. lipolytica* 17/184(9.2%), *C. orthopsilosis* 4/184 (2.2%), *C. zeylanoides* 4/184 (2.2%), *C. glabrata* 3/184 (1.6%), *C. famata* 3/184 (1.6%), *C. dubliniensis* 2/184(1.1%), *C. tropicalis* 2/184(1.1%), *C. lusitanae* 1/184 (0.5%), *C. catenulata* 1/184 (0.5%), *C. pelliculosa* 1/184 (0.5%), *C. krusei* 1/184 (0.5%), *Candida* spp. 9/184 (4.9 %).

**Conclusions.** In Latvia most common dermatophytes were *T.rubrum* (82%), *T. mentagrophyte* (6.4%), *T. tonsurans* (4.4%) and *M. canis* (3.8%). *T. rubrum* had emerged as the predominant global agent, with a prevalence of over 40-70% in Central and North European countries, followed by *T. mentagrophytes*. In 2023 year most common yeasts were *C. parapsilosis* (42.4%) and *C. albicans* (21.7%).

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## Detection of *Bordetella pertussis* disease in National Reference Laboratory in Latvia in 2022-2024 years

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**Keywords:** *Bordetella pertussis* Whooping cough Pertussis.

**Introduction.** Whooping cough is bacterial respiratory infection disease caused by *Bordetella pertussis*. Pertussis remains endemic worldwide and tends to be a cyclic disease, peaking every 3-5 years. Laboratory methods (ELISA, PCR or microbial isolation) give different results depending on the stage of infection.

**Aims and Objectives.** The aim of study was to evaluate effectiveness of different diagnostic methods: microbial isolation, serology and RT-PCR testing in laboratory diagnosis of *Bordetella pertussis*.

**Material and Methods.** Laboratory confirmation of children and adults pertussis cases in Latvian NRL was done by positive *B. pertussis* Toxin IgA ELISA in single sera, rise of *B. pertussis* Toxin IgG antibodies in paired sera detected by ELISA and/ or positive result in *B. pertussis* DNS detection with rt-PCR method and/or microbial isolation in growth media in dry throat or nasal swabs, or swabs in transport medium. Data about beginning of clinical symptoms and vaccination status were not provided to laboratory.

**Results.** 393 samples were tested for IgA (35 positive, 8.9%), 421 samples for IgG (36 positive, 8.6%); 156 samples were tested for *B.pertussis/B.parapertussis* multiplex RT-PCR (25 positive for *B.pertussis*, 16%). Also tested were 2473 samples for multiplex assays for bacterial pneumonia or respiratory disease (i.e. physicians did not suspect *pertussis* specifically), obtained more 5 positive results for *B.pertussis*. Of the 25 positive samples sent for *B.pertussis/B.parapertussis* multiplex RT-PCR, 15 patients had blood sampled for antibodies to *B.pertussis* at the same time, but all results were negative. After 2-4 weeks, blood was taken again from the same patients for antibodies and the result was positive for all patients who had previously been positive by RT-PCR. From these data we can assume that RT-PCR is better at diagnosing the early stages of the disease, when the antibodies have not yet had time to form. Only 9 samples were sent for bacterial culture, but the result was negative in all cases.

**Conclusions.** PCR demonstrated possibility of earlier diagnosis comparing to serology especially in children. Interpretation of laboratory results may be done only in context with vaccination status and data ab beginning of clinical symptoms.

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## Glycated hemoglobin and cholesterol testing in Latvian population – single laboratory data

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**Keywords:** glycated hemoglobin, cholesterol, laboratory testing.

**Introduction.** Glycated hemoglobin (HbA1c) and cholesterol are extensively used for prophylaxis, diagnostics and monitoring and may serve as indicators for overall quality of medical services.

**Aims and objectives.** To study testing coverage and rate of abnormal results by gender and age of HbA1c and cholesterol in a bulk single laboratory cohort.

**Materials and methods.** Results of HbA1c and total cholesterol tests performed at SIA "Centrālā laboratorija" in 2022 on ARCHITECT i4000SR were analyzed. Demographic structure of Latvian population was obtained from the Latvian Central Statistical Bureau and split into 10-year age groups. Yearly testing coverage (percentage of age group population tested) and prevalence of abnormal tests (percentage of abnormal results from tests in age groups) were calculated.

**Results.** HbA1c. 88596 tests, M:F 0.60. 60.1% results abnormal (99.4% elevated), 62.1% in males and 58.7% in females. Testing peak in females at 70-79 (coverage 13.6%) and broad plateau of abnormal results at 60-89 (abnormal rate above 65%). Testing intensity in males also the highest at 70-79 (11.7%), while abnormal results peaked at 60-69 (72.0%) and remained high. Percentage of abnormal results after 80 in both genders only slightly lower than peak values, but testing dropped almost to zero. Cholesterol. 290231 tests, M:F 0.62. 60.2% results abnormal (88.5% elevated), 54.9% in males and 63.5% in females. In females, maximal testing was at 60-69 (coverage 25.3%), while the peak of abnormal results was at 50-59 (77.5%). In males, the most covered age group was also 60-69 (coverage 24.1%), but the peak of abnormal results was at 40-49 (69.1%). The rate of abnormal results decreased in males after 80, but remained high in elderly females (about 50%); coverage dropped dramatically in both genders.

**Conclusions.** The study revealed a potential for improving the studied regimens. HbA1c test count was more than 3 times lower than cholesterol, particularly in children, younger adults and elderly; the result of relying on fasting glucose for diabetes diagnostics and monitoring. Testing for cholesterol is insufficient in younger adults and seniors. Increase of abnormal cholesterol results becomes apparent after age 30 and abnormal HbA1c after 40, supporting early screening for dyslipidemia and diabetes. "Centrālā laboratorija" is the largest laboratory in Latvia, nevertheless, the study is clearly limited. Comparison with other Latvian and international data would be useful.

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## Testosterone level in a large male cohort – implications for defining normal range

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**Keywords:** testosterone, laboratory testing, age variations.

**Introduction.** Testosterone is the key male hormone, nevertheless, there are only limited data on its age-related dynamics and diurnal and seasonal variations. Age-related increase in boys is a recognized phenomenon, but adults irrespective of age are considered homogenous cohort in laboratory practice; that makes distinction between physiological age-related decrease from late hypogonadism problematic.

**Aims and Objectives.** The aim of the study was to analyze age, seasonal and diurnal variability of testosterone level in a bulk cohort of male outpatients.

**Materials and methods.** Continuous results of 10945 ambulatory male total testosterone tests performed in 2015-2021 at SIA "Centrālā laboratorija" (ARCHITECT i4000SR) were anonymously retrieved and analyzed by IBM SPCC v.25.

**Results.** In the studied cohort, mean testosterone level was near zero at age 0-10 (mean 0.15 ng/mL, 5th - 95th percentile 0.02-0.25 ng/mL). Transition period was at age 11-14 (2.48, 0.12-7.11 ng/mL), overt increase at 15-17 (5.23, 1.63-9.54), peak at 18-40 (7.45, 2.04-16.50), decrease at 41-55 (5.69, 1.44-10.70) and plateau after 55 (3.39, 0.13-8.82 with lower norm near zero). At age 18-40, 95th percentile was lower in the morning (Kruskal-Wallis  $p < 0.01$ ) and dropped in September-December ( $p < 0.01$ ). Variation in other age groups were insignificant. When applying manufacturer reference range of 2.3-10.2 ng/mL, 27.70% adult tests were abnormal: 6% low and 9% high at age 18-40, 11% and 6% at 41-55, 41% and 2% at >55.

**Conclusions.** The studied population was not exactly normal, still, the cohort is sufficient for preliminary inferences. The obtained 5th-95th pediatric percentiles at age 0-17 match previously published series (Cohen, 2020; Baum, 2020) and could be tentatively used as reference. The study demonstrated that testosterone levels significantly decreases with age. To assure proper result interpretation, at least 3 adult age groups should be separated: 18-40 (peak level), 41-55 (decrease of upper norm) and >55 (decrease of lower norm as well). This approach allows to reduce the unreasonable 41% of abnormally low results in elders and reclassifies half of the elevated results at 18-40. Further studies and clinical validation would be necessary to confirm the findings. The found diurnal and seasonal variations may be significant for optimal testing regimen and for result interpretation.

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## HPV prevalence among women attending cervical screening in Latvia in 2023 year

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**Keywords:** high risk papilloma virus infection, HPV screening, Latvia.

**Introduction.** Human papilloma virus (HPV), which is the causative agent of cervical cancer, is one of the most common viral infections of the reproductive system and can be easily diagnosed. To reduce the incidence of cervical cancer, a state-funded nationwide screening program is of great importance. According to the Global Strategy and general recommendations of the World Health Organization, high-risk HPV type DNA testing by highly specific and sensitive molecular diagnostics methods is recommended.

**Aims and Objectives.** Preventive cervical cancer screening program is realized in Latvia for women aged 25-67. Data on the women who have responded to national screening program in Latvia were gathered in a Central laboratory (Riga, Latvia) from the 1st of January 2023 until the 31st of December 2023.

**Materials and Methods.** 14 high-risk HPV types DNA testing was done by Cobas 6800, Roche. Testing is based on real-time PCR and the system detects and differentiates HPV 16, 18 types and makes a pull of other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68).

**Results.** Overall, during 2023 year Central laboratory performed 58911 high-risk HPV DNA tests. It was calculated that 11.5% of the received samples were positive to at least one high risk-HPV type. HPV 16 type was positive in 2.5% cases, HPV 18 type in 0.8% following other high-risk HPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 or 68) with the highest positive cases found - 8.2%. These single-type HPV infections are more common than multiple-type HPV infections. The overall calculated co-infection rate was 1% in the analyzed samples in a given period of time. Globally, the high-risk HPV positivity rate varies between countries, however the current global HPV prevalence is estimated to be 11.7%.

**Conclusions.** Testing data indicates that the prevalence of high-risk HPV types in Latvia is comparable with the global average. It is crucial to maintain and promote the national cervical cancer screening program that includes broad testing. Testing with molecular diagnostics methods provides valuable statistical data that helps evaluate the prevalence of high-risk HPV in Latvian population in the upcoming years.

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## Screening for common BRCA1/BRCA2 pathogenic variants using in-house SNaPshot panel

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**Keywords:** BRCA1, BRCA2, breast cancer, ovarian cancer, SNaPshot.

**Introduction.** To date, the most important and best characterized genetic risk factors for breast and ovarian cancer are germline pathogenic variants (PV) in the BRCA1 and BRCA2 genes. The lifetime risk of developing breast and ovarian cancer in PV carriers varies between studies and is related to variant location and family history. The risk of developing breast and ovarian cancer by the age of 70 years has been reported to be 45-85% in BRCA1 PV carriers and 27-84% in BRCA2 PV carriers. The prevalence of BRCA1 and BRCA2 PV carriers in the general population is approximately 0.2%, but this may vary considerably by variant, between countries or between some ethnic groups due to founder effect. Testing for population specific founder PV could be reasonable in countries where full BRCA1 and BRCA2 gene sequencing and copy number variant detection is not reimbursed by country. Here we are sharing our experience in long term screening for Latvian frequent founder PV of BRCA1 and BRCA2 by our own laboratory developed test.

**Aims and Objectives.** Develop a good test in the current economic situation in Latvian healthcare that will be able to detect the largest number of frequent PV in the population.

**Materials and Methods.** SNaPshot, Sanger sequencing, statistical analysis.

**Results.** SNaPshot (minisequencing) panel was developed for detection founder BRCA1 and BRCA2 PV specific to Latvian population according to published data in 2016. Panel included 11 PV – Latvian founder PV, common population PV. Recently (in 2024) panel was updated according to new data for common Latvian PV and now detects 20 variants. On the day of submission of the thesis, 4442 women and 74 men were tested using both tests (old and new). Overall, 7.43% of women tested positive for one of the PV. Our laboratory database does not always include a specific diagnosis, but in general, a panel of BRCA1 and BRCA2 founder PV is usually performed in women patients with ovarian or breast cancer. The BRCA1 and BRCA2 PV positive rate in breast and ovarian cancer varies between studies using NGS panels, depending on cancer type and cohort selection, and is typically in the range of 9-12%.

**Conclusion.** In the current economic situation in our healthcare, an appropriate test has been developed that can give results relatively quickly and at low cost. The methodology allows for modifications – reducing or expanding the number of variants to be tested, and confirming suspicious results using Sanger sequencing.

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## The differences in complete blood count, biochemical and coagulation test results between high and low hepatitis C viremia subjects

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**Keywords:** HCV, viremia, CBC, biochemical, coagulation.

**Introduction.** Hepatitis C viral infection (HCV) is a serious global public health problem that affects approximately 1% of the world's population. The World Health Organization (WHO) has outlined a set of targets to eliminate hepatitis C by 2030. In 2022, Lithuanian authorities initiated an HCV screening program to eliminate HCV, thus detecting possible HCV-related deaths, cases of hepatocellular carcinoma, and decompensated cirrhosis. The aim was to evaluate CBC, biochemical, and coagulation tests in high and low HCV viremia.

**Material and Methods.** The 216 random subjects who participated in a screening program for HCV infection were included in the study-151 subjects (95M/56F) with high viremia (>800000 cop/mL) as hiVG, and 65 subjects (27M/38F) with low viremia (<800000 cop/mL) as loVG. All subjects were anti-HCV positive. Viremia was evaluated using qRT-PCR, and the genotype was assessed using VERSANT HCV Genotype 2.0 Assay Kit, CBC - BC UNICel DxH 800 analyzer, biochemical tests - BC AU680 Clinical Chemistry Analyzer, and coagulation tests - Stago STA R Max analyzer.

**Results.** The results showed that there were some significant differences between hiVG and loVG in biochemical, CBC, and coagulation results: - CBC - RBC, HGB, HCT, WBC, PLT, and PCT results had significant differences between groups,  $p < 0.05$ ; Biochemical tests like ALP, DBIL, TBIL, and ALB significantly differed between hiVG and loVG,  $p < 0.05$ ; -Coagulation tests - SPA (Owren's method), by sec., %, and INR are also significantly different,  $p < 0.05$ . Then, the hiVG and loVG were separated by gender into four groups. Results showed that the differences in males hiVG and loVG groups were the same as in the general subjects' pool; however, in females, differences were found only in RBC, HGB, and ALB,  $p < 0.05$ . When comparing results to the normal range, we found that: - males hiVG had lower PLT, RBC, and PCT results but higher ALT, GGT, and AST; - males loVG had lower SPA(%), PCT, PLT results, but higher SPA(sec.), ALT, AST; - females hiVG had lower PLT, PCT, and SPA INR results but higher AST, ALT, and GGT; - females loVG had lower PLT, PCT, and ALB results but higher SPA (sec.), SPA(%), and GGT.

**Conclusions.** Active infection with the hepatitis C virus, depending on the gender and viremia level, changes the results of CBC, biochemical, and coagulation tests. Therefore, we recommend a complete workup of patients when HCV viremia is detected.

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## Influence of non-refrigerated urine storage time on stability of urine particles

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**Keywords:** urinalysis, specimen handling, automated urine particle analyzer.

**Background.** The EFLM European Urinalysis Guidelines provide clear instructions for urinalysis. Regarding time for particle examination, it is recommended that the specimen for particle counting should be refrigerated if not examined within 2–6 h and noted, that WBC counts may be questionable after 2–4 hours, even with sample refrigeration. Labs can reliably follow recommendation for the first morning urine examination only for in-patient samples and with limitations for out-patient samples, only when patient lives close to the laboratory. Individual portable refrigerators for urine transportation are not available for patients in Estonia. Thus recommended first morning urine analysis is not possible for majority of out-patients as samples are discarded at preanalytical department or quality of analysis is not assured because labs analyze “delayed” samples. We investigated the effect of urine storage time on the stability of urine particles.

**Materials and Methods.** Urine samples were collected using BD Vacutainer Urinalysis Tube. The 63 urine samples from different hospital departments were analyzed immediately and within 6 hours (+/- 15 min) after arrival at the laboratory by image-based analysis systems: Atellica UAS 800 or Cobas 6500 (u601+u701). All samples were stored at room temperature. Both analytical platforms obtained 15 images of each sample using an integrated microscope and digital image processing software detected and classified urine particles. The results were expressed as average of formed elements/particles per HPF.

**Results.** All results were categorized according to relevant clinical decision: According to the AUA/SUFU guidelines, the number of RBCs in urine sample plays crucial role in determining the genitourinary malignancy risk category: 3–10 RBC/HPF for low-risk, 11–25 RBC/HPF for intermediate-risk, and >25 RBC/HPF for high-risk classification. Urine specimen with evidence of pyuria were defined as at least 10 WBC/ $\mu$ L (2.27 WBC/HPF). However,  $\geq 10$  WBC/ $\mu$ L in urine suggests the presence of UTI, but is not a diagnostic criterium. No clinically significant changes in results interpretation for RBC, WBC, HYA, PAT, NEC, EPI/SEC, YEA, BAC and CRY were observed after 6 h of storage. All negative results remained negative and positive (hematuria, pyuria) remained positive. No lysis of casts, epithelial cells or crystals was present for 6 hours.

**Conclusions.** Results of our study demonstrate there are no clinically significant changes of urine particles in non-refrigerated urine samples for up to 6 hours.

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## SARS-CoV-2 spike-specific B cell responses in chronic lymphocytic leukaemia

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**Keywords:** B cells, Chronic lymphocytic leukaemia (CLL), SARS-CoV-2 vaccine response.

**Introduction.** Chronic lymphocytic leukaemia (CLL) is associated with secondary immunodeficiency and infections remain the major cause of mortality in CLL patients. Disease-driven immune dysfunction and immunosuppressive therapies have been suggested to underly this immunodeficiency. Nevertheless, B cell composition in CLL patients has not been characterized and it is not known how it impacts antigen-specific B cell differentiation, such as that to SARS-CoV-2 vaccination.

**Aim.** To characterise the overall non-malignant B cell landscape as well as the development and phenotype of SARS-CoV-2 spike protein-specific B cells following vaccination in CLL patients compared to healthy controls (HCs).

**Materials and Methods.** We recruited CLL patients who met the WHO 2017 and the International Workshop on CLL diagnostic criteria; CLL patients were either treatment naïve (CLL-TN) (n=23) or chemotherapy-receiving (CLL-CTx) (n=11). The control group (HC, n=16) was composed of age- and sex-matched individuals. All study participants received at least 2 doses of SARS-CoV-2 vaccine and time post-vaccination was comparable between groups. Using flow cytometry, we phenotyped B cells, including SARS-CoV-2 spike protein-specific, among peripheral blood mononuclear cells. Anti-SARS-CoV-2 spike IgG levels were determined by ELISA. The data were analysed with GraphPad Prism 10. Results were considered statistically significant at  $p < 0.05$ .

**Results.** Despite the considerable expansion of the malignant B cells and prominent alterations in peripheral B cell subsets in patients with CLL, the absolute numbers of circulating SARS-CoV-2 spike-specific B cells were comparable between CLL-TN, CLL-CTx and HCs. Furthermore, when interrogating the phenotype of spike-specific B cells, approximately half were IgG-class-switched in all groups, suggesting preserved B cell differentiation pathways to SARS-CoV-2 vaccination in CLL. That B cell response to SARS-CoV-2 spike protein was maintained, was supported by anti-SARS-CoV-2 IgG levels that were comparable between groups.

**Conclusions.** These data suggest that despite the major alterations in B cell subsets in CLL patients, the ability of the B cell compartment to give rise to SARS-CoV-2 spike-specific B cells is preserved in patients with CLL.

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## Fungal infection agents microbiological examination in comparison with direct microscopys

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**Keywords:** Fungal cultures, direct microscopy, dermatophytes.

**Introduction.** Skin and its derivatives fungal infections are common worldwide. According to WHO data, an average of 300 million people suffer from fungal infections every year. The main risk factors for contracting dermatomycoses are immunodeficiency, chronic diseases, menopause, long-term use of antimicrobial agents, skin and nail damage, age. Statistics show that more likely older people suffer from infections caused by dermatomyces. In the laboratory diagnosis of fungal infections, the most often used methods are direct microscopy, microbiological examination and in recent years molecular biological investigation methods.

**Aims and Objectives.** The aim of this study was to collect and compare data of the most used methods in laboratory practice: microbiological investigation and direct microscopy. Materials and methods. In the period from August 2022 to August 2023, Central Laboratory LTD conducted parallel examination of 2,785 patient samples (skin, mucous membranes, nails) culturing and identification and direct microscopy.

**Results.** Out of 2785 samples, 1656 (59.46%) microbiological examinations and 670 (24.06%) direct microscopies were positive for dermatomycosis agents. Out of 1656 positive microbiological examinations, fungal elements were found in 411 (24.81%) samples by the direct microscopy. From 1129 (40.54%) negative samples by microbiological examination in 259 (22.94%) fungal elements were found by the direct microscopy. The most often isolated pathogens were *Trichophyton* sp., *Geomyces* sp., *Candida* sp., *Aspergillus* sp., *Scopulariopsis* sp. and *Exophiala* sp.

**Conclusions.** Microbiological examination is a more sensitive and informative method for the laboratory diagnosis of infections caused by dermatomyces, but the investigation time is longer than with the direct microscopy method. Due to large numbers of negative fungal cultures, but positive direct microscopy findings, it is preferable to do cross examination with both methods. Due to the substantial number of negative samples, it is desirable to apply molecular diagnostics more widely.

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## Diversity of antibiotic resistant phenotypes among isolates from periprosthetic joint infections

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**Keywords:** periprosthetic joint infections, antimicrobial resistance, MDR.

**Introduction.** Periprosthetic joint infections (PJI) are severe complications that can compromise the success of joint replacement surgeries.

**Aims and Objectives.** This study aims to evaluate the causative microbial agents of PJIs and their resistance phenotypes, providing critical insights into the prevalence of antibiotic-resistant strains.

**Materials and Methods.** Microorganisms were identified from PJI samples by Vitek 2 compact system, antibiotic susceptibility was evaluated by using Kirby-Bauer disk diffusion method. Epidemiologically relevant resistance phenotypes were confirmed by phenotypic (NG-Test Carba 5, combination disk method for ESBL) and genotypic tests (BIOFIRE JI Panel, BIOFIRE BCID2 Panel). Data were derived from the laboratory information system and isolated bacterial strains were phenotyped according to antibiograms. Data processed using MS Excel 2021. Analyzed results were obtained from positive cultures from patients with confirmed periprosthetic joint infections in 2022 and 2023 at the Hospital of Traumatology and Orthopedics.

**Results.** The study analyzed a total of 122 cases with PJI (hip n = 73; knee n = 43, shoulder n = 6) were included, of which 55.7% were women and 44.3% were men, aged 34 - 95 years. Average age 69.3 years. The most common causative pathogens were gram-positive cocci (73.22%, n=93). *S. aureus* (n = 43; 33.85%) was the most frequently detected, followed by  $\beta$  - hemolytic *streptococci* (n = 18; 14.17%) and *S. epidermidis* (n = 17; 13.38%). Gram-negative pathogens and fungi could be detected in 26.8% (n = 34) and 1.6% (n = 2) of all cases. The prevalence of polymicrobial growths was (n = 11; 9.01%) The percentage of MDR isolates was 18.1% (n=23) Methicillin resistance was 3,22% for *S.aureus* and 7.52% for *S. epidermidis*. ESBL was detected in 20.5%, n=7. There were 14.7% (n=5) carbapenemase producing isolates. The most common gene identified in MDR pathogens was *mecA/C* (n=10, 50%), carbapenemase genes were detected in five isolates *blaVIM* (n=2) *blaNDM* (n=2), *blaOXA* (n=1). Among gram-negative bacteria highest resistance rates were against cefazolin (47.05%), gram-positive isolates showed the highest resistance to gentamicin (22.58%), doxycycline (17.2%) and erythromycin (15.05%)

**Conclusions.** Antimicrobial resistance surveillance among PJI isolates under the antimicrobial stewardship program reveals high rates of resistance. Continuous surveillance is essential for managing resistant PJI pathogens and improving patient outcomes.

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## Evaluating Patient Preparation Practices for Blood Tests Among Healthcare Professionals in Lithuania: A Cross-Sectional Study

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**Keywords:** Patient preparation, quality of healthcare, healthcare practices, preanalytical phase.

**Introduction.** Accurate laboratory test results strongly depend on proper patient preparation, an often-underestimated aspect of diagnostics. Consistent and clear instructions from healthcare professionals are crucial for maintaining the quality of laboratory testing.

**Aims and Objectives.** This study aims to assess the practices of physicians and nurses in Lithuania regarding patient preparation for blood tests. The objective is to identify variations in practices and propose enhancements to improve the accuracy of laboratory results.

**Materials and Methods.** A survey was conducted between February and October 2023 using an electronic questionnaire distributed to 141 public healthcare institutions in Lithuania. Participants were asked about their practices in preparing patients for laboratory tests.

**Results.** Among 113 respondents (75 nurses, 38 physicians), 58% of physicians and 71% of nurses adhered to laboratory-prepared guidelines. Verbal instructions were given by 56% of physicians and 51% of nurses, with limited use of written materials. Fasting recommendations included a 12-hour period before blood sampling, supported by 73% of physicians and 84% of nurses. For caffeine, 67% of physicians and 77% of nurses advised against intake before collection, while 55% of physicians and 77% of nurses recommended 24-hour alcohol abstinence. Additionally, 42% of physicians and 63% of nurses advised refraining from smoking for an hour before sampling. Recommendation against non-essential medications was provided by 55% of physicians and 74% of nurses. Physical activity recommendations were conservative, with only 15% of physicians and 26% of nurses advising against vigorous exercise the night before testing. Notably, 45% of nurses and 42% of physicians emphasized the importance of patients informing blood collection staff about the day of their menstrual cycle, especially during hormone testing. Additionally, 58% of physicians and 52% of nurses advised scheduling blood tests before radiological procedures involving contrast agents. Finally, 18% of nurses and 12% of physicians recommended refraining from water intake before sampling.

**Conclusion.** The study reveals disparities in patient preparation practices between physicians and nurses. While adherence to guidelines is generally observed, variations, especially concerning alcohol intake and medication guidance, are evident. Standardization of patient preparation practices could improve the accuracy of laboratory outcomes.

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## Initial evaluation of the Ogata score in routine laboratory practice

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**Keywords:** Myelodysplastic syndrome, Ogata score, flow cytometry.

**Introduction.** Myelodysplastic syndromes (MDS) is a heterogeneous group of clonal hematopoietic stem cell neoplasms. It is characterized by ineffective hematopoiesis, peripheral blood cytopenias and increased risk of leukemic evolution. However, diagnosis may be challenging. Ogata score (OS) is a simple flow cytometry-based tool that can be implemented in the diagnostic workup for MDS.

**Aims and Objectives.** To evaluate the OS performance in patients with suspected hematological malignancies at Pauls Stradins Clinical University Hospital's Joint Laboratory.

**Materials and Methods.** OS was calculated in routine bone marrow samples sent for hematological disorder immunophenotyping. OS included assessment of neutrophils' side scatter, percentage of myeloblasts and B cell progenitors, evaluation of CD45 and CD7 expression on myeloblasts and CD56 on monocytes. Ogata score  $\geq 2$  was considered positive. Data from 17 patients were analyzed between January and May 2024. OS were correlated with results from histological analysis.

**Results.** Bone marrow biopsy showed that three of the 17 patients had MDS, 1 was inconclusive with suspicion for MDS, 7 - multiple myeloma, 3 - acute myeloid leukemia (two of them had progressed from MDS). The remaining 3 patients had reactive changes in the bone marrow. Ogata score was positive in 9 patients: all 3 confirmed MDS, 1 myeloma case (in this case biopsy was suboptimal and normal hematopoiesis was not evaluated), all three acute leukemias and in 2 patients with reactive changes (one of these also had suboptimal biopsy material).

**Conclusions.** Initial data shows promising results of OS as an additional test for MDS diagnostic workup. Still, more data is needed for the OS performance verification.

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## Ascites as the first presentation of plasmablastic lymphoma in HIV positive individual

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**Keywords:** Plasmablastic lymphoma, HIV, ascites, B cells.

**Introduction.** Human Immunodeficiency Virus (HIV) induced weakening of the immune system leads to increased frequency of lymphomas in HIV infected persons. Plasmablastic lymphoma (PBL) is a rare, aggressive post-germinal center B cell neoplasm that represents approximately 3% of HIV related lymphomas.

**Aims and Objectives.** Here we describe a case of HIV positive patient who presented to the emergency department with ascites of uncertain etiology.

**Materials and Methods.** Cytological, immunophenotypic and bacterial culture studies was done in ascitic fluid collection from 59 years old male. Flow cytometric analysis was done stepwise with markers: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD15, CD16, CD19, CD20, CD33, CD34, CD38, CD43, CD45, CD57, CD138, HLA-DR, surface and cytoplasmic kappa and lambda. In addition, radiographic and histological evaluation was done.

**Results.** Analysis of ascitic fluid showed cytositis 1400 cells/mkl with 96% lymphocytes. Bacterial studies were negative. Morphological evaluation revealed atypical lymphocytes with abundant basophilic cytoplasm with vacuoles, pleomorphic nuclei and nucleoli. Flow cytometry evaluation indicated atypical population composed of large cells with increased forward scatter, negative for all evaluated markers except CD43, CD138, partial expression of CD7, CD56 and dim expression of CD38 and CD45. Peripheral blood count indicated absolute lymphopenia. Mediastinal and peritoneal lymphadenopathy was detected on computed tomography. A histomorphology from endoscopically suspicious duodenal ulcer confirmed diagnosis of PBL.

**Conclusions.** Initial presentation of PBL as an ascites is a rare finding. Furthermore, diagnosis of PBL is challenging – cytomorphological features of neoplastic cells resemble immunoblastic variant of diffuse large B cell lymphoma, but phenotype shows lack of B cell markers and expression of plasma cell antigens. Appropriate correlation of clinical, laboratory and radiographic findings are crucial in establishing the correct diagnosis.

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## Clinical Utility of von Willebrand Factor Multimers Assay

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**Keywords:** von Willebrand disease, multimers, desmopressin, von Willebrand factor, electrophoresis.

**Introduction.** Von Willebrand disease is one of the most common blood coagulation disorders. The diagnosis of this disease is challenging and complex, requiring numerous tests. One of the diagnostic challenges is the analysis of von Willebrand multimers using gel electrophoresis. Due to the high costs involved, there is a question regarding the justification of performing this test for all patients exhibiting symptoms of excessive bleeding.

**Objective.** To evaluate the clinical utility of von Willebrand factor multimer analysis based on available results of von Willebrand factor multimers, antigen, activity, factor VIII, and patient coagulometric results.

**Material and Methods.** Out of 143 subjects, 83 patients were selected for whom von Willebrand factor multimer electrophoresis, von Willebrand factor activity, and antigen concentration were determined. The activity/antigen ratio was calculated for these patients.

**Results.** A changed pattern of multimer fractions was observed in 28.9% of cases. The vWF:Ac/vWF:Ag ratio was < 0.7 in 10.8% of cases. Among all subjects with a vWF:Ac/vWF:Ag ratio > 0.7, 7 (8.4%) showed a deficiency in HMW. There is a moderate correlation between the concentration of LMW multimers in the blood and vWF:Ag and vWF:Ac as well as their ratio. A moderate correlation exists between the concentration of IMW multimers in the blood and vWF:Ag and vWF:Ac, with no observed changes in their ratio. Changes in HMW multimer concentrations have no significant association with vWF:Ag, vWF:Ac, or their ratio changes. An increase in the intensity of HMW and IMW multimer fractions is observed one hour after DDAVP administration, with a decrease in these fractions intensity observed after four hours.

**Conclusions.** According to the guidelines vWF:MM analysis should be performed for patients whose vWF:Ac/vWF:Ag ratio is < 0.7. Among the studied patients, this ratio was < 0.7 in 10.8% of cases. Of the 83 patients with a vWF:Ac/vWF:Ag ratio >0.7, 8.4% showed a reduced intensity of the HMW multimers fraction. The concentration of LMW and IMW multimers in the blood depends on vWF:Ag and vWF:Ac levels, while these indicators do not affect the concentration of HMW multimers. vWF multimer analysis allows monitoring the effect of DDAVP in patients with von Willebrand disease.

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## Presence of point mutations A2063G and A2064G in the DNA of patients with *Mycoplasma pneumoniae*

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**Keywords:** *Mycoplasma pneumoniae*, point mutation, resistance.

**Objectives.** *Mycoplasma pneumoniae* is a small pathogenic bacterium without a cell wall that can cause atypical pneumonia in humans. Cultivation of *M.pneumoniae* is technically difficult and often unavailable, but laboratory testing is possible using Polymerase Chain Reaction (PCR) tests or/and antibody detection. Primarily *M.pneumoniae* is treated with macrolide antibiotics, however resistance to the macrolides has been observed and it is usually associated with point mutations in the V domain of the 23S rRNA of *M.pneumoniae*. A2063G or A2064G mutations confer the highest resistance to macrolides.

**Materials and methods.** In the period 08.2023 - 05.2024 during the routine real-time PCR testing for the presence of bacterial respiratory pathogens (Seegene Allplex PneumoBacter Assay) 37 DNA (16 nasopharyngeal swabs; 12 bronchoalveolar lavages (BAL); 9 sputums) of 33 patients had *M.pneumoniae* DNA. They were examined for A2063G and A2064G mutations using Sanger sequencing protocol. The 200-bp PCR product of domain V of the 23S rRNA gene was amplified and then the sequencing reaction was performed with the BigDye 3.1 terminator cycle sequencing kit. Consensus sequences were aligned with MEGA11 software.

**Results.** From 37 *M.pneumoniae* positive DNA samples obtained from 33 patients, three of whom had more than one material tested (two patients had 2 respiratory samples each and one patient had 3 samples for testing). The results obtained were 100% identical when testing different materials on the same patient. We found a point mutation A2063G in 12 (36%) patients. 2 clones (wild and mutant) of *M.pneumoniae* were observed at positions 2063 and 2064 in one patient. There were 6 samples of BAL, 5 of them were sputum and 4 were nasopharyngeal swabs. The majority of patients with infection caused by *M.pneumoniae* was in the age group 21-40.

**Conclusions.** Timely diagnostic of infection caused by resistant *M. pneumoniae* is crucial to avoid complications during treatment and shorten treatment time. The use of different testing materials for the same patient allowed us to validate these materials and check the reliability of the results. Knowing that the Sanger sequencing method takes several days, the next stage of our research will be to develop a PCR screening protocol using these positive validated samples.

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## Application of Whole-Genome Sequencing for Distinguishing Relapse from Reinfection in Tuberculosis Patients from Lithuania

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**Keywords:** Tuberculosis, relapse, reinfection, whole-genome sequencing.

**Introduction.** Distinguishing the cause of tuberculosis (TB) recurrence is crucial for tracing transmission chains and guiding timely treatment. TB reinfection can be mistaken for a relapse or treatment failure due to a changed drug resistance pattern after initially successful treatment. TB reinfection could likely occur in the household, within the community, or even in the hospital ward during treatment. The actual rate of TB reinfection in Lithuania has never been reported, and the reasons for TB recurrence remain poorly understood.

**Aims and Objectives.** The aim of this study was to assess the rate of true TB relapses versus reinfection among patients with TB recurrence in Lithuania using whole-genome sequencing (WGS).

**Materials and Methods.** The study included 62 Mycobacterium tuberculosis isolates, recovered from as many TB patients who had at least one reported TB relapse (20/29; 69%) or treatment failure (9/29; 31%) episode and were treated in Vilnius University Hospital Santaros Klinikos between 2016 and 2023. WGS was performed with Illumina NovaSeq 6000 sequencer in 2x150 bp paired-end mode. The multiple sequence alignment of alleles was constructed using the MTBseq v1.0.3 bioinformatic pipeline, setting the distance at 5. To differentiate between relapse and reinfection, a cut-off of 5 single nucleotide polymorphisms was used. Subsequently, Randomized Accelerated Maximum Likelihood (RAxML) v.8.2.12 was used to produce a robust bootstrap-supported phylogenetic tree. The resulting phylogenetic tree was visualized and annotated using the online interface Interactive Tree of Life (iTOL).

**Results.** The most prevalent genotype was Beijing (2.2.1) (30/62; 48.4%), followed by LAM (4.3.3) (15/62; 24.2%), mainly T (4.8 and 4.7) (10/62; 16.1% and 1/62; 1.6%, respectively), Ural (4.2.1) (5/62; 8.1%), and H37Rv-like (4.9) (1/62; 1.6%). The majority (12/20; 60%) of all reported relapses were caused by true relapse, while reinfections accounted for 40% (8/20). Of 9 patients reported as treatment failures, 4 were reinfections, 1 was both reinfection and treatment failure and only 4 were true treatment failures.

**Conclusions.** Only 55% (16/29) of patients were correctly differentiated as relapse or treatment failure by clinicians. A high rate of misclassified reinfections as relapse or treatment failure due to limited diagnostics poses a challenge to TB control efforts. Thus, the implementation of WGS may improve timely diagnosis and TB control in Lithuania.

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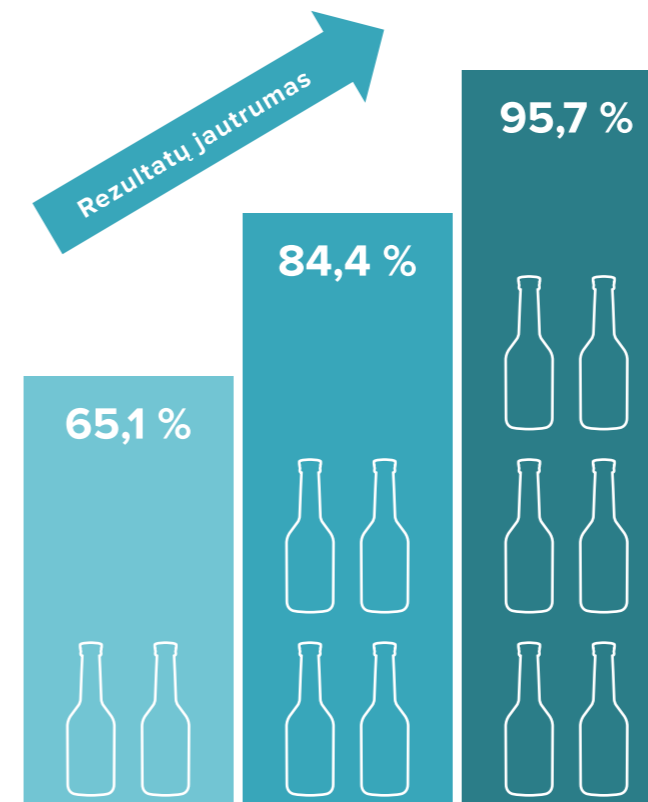


Ankstyvas sepsio atpažinimas  
ir gydymas **gelbsti gyvybes**

# Sepsio diagnostikos gairės kraujo pasėliams

Pagal Europos klinikinės mikrobiologijos vadovą (SFM/ESCMID) suaugusiesiems pacientams paimama:

- ✓ nuo 4 iki 6 kraujo pasėlių buteliukų, t.y. 40-60 ml kraujo arba
- ✓ 2-3 rinkiniai iš skirtingų kūno vietų<sup>3</sup>.



Remiantis Queensland health organizacijos duomenimis.



**BACTEC™ Mycosis-IC/F**  
Tai selektyvi terpė grybinėms kultūroms augti, slopinanti bakterijų augimą. Naudodami šią terpę galite diferencijuoti fungemines ir mišrias infekcijas. Diagnostinis pranašumas - 57,7 % iš tirtų atvejų tai buvo vienintelis teigiamas buteliukas iš rinkinio (anaerobinio, aerobinio ir grybinių kultūrų)<sup>1</sup>.



**BACTEC™ Lytic**  
Anaerobinė terpė yra sukurta siekiant padidinti anaerobų aptikimą. Šioje terpėje yra detergentas, skirtas raudoniesiems ir baltiesiems kraujo kūneliams lizuoti, išlaisvinant bet kokį viduląstelinį organizmą. Aptikimas buvo žymiai didesnis (iki 94 %) nei kitų rinkoje esančių terpių<sup>2</sup>.

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