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Laboratory diagnostics of blood-borne infections related to blood transfusions and transplantations

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Croatian Institute of Transfusion Medicine

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Epidemiological characteristics of HIV, HBV, HCV, syphilis, CMV, EBV and Toxo in Croatia

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All infective diseases having a viremic phase can be transmitted via blood. However in the majority, this risk is routinely eliminated by avoiding febrile and manifestly ill persons as well as convalescents as blood donors. A challenge represent the infections tending to become chronic, and asymptomatic but still having permanently or intermittently the causative agents in blood. A scientifically founded and rational tactics of routine blood testing and aimed questioning of donors regarding their clinical and epidemiological anamnesis is crucial here in reducing transfusion infection risks to a minimum. Important influence has also the domestic epidemiological situation. According to wishes of the organizers, this article presents the current status and epidemiological characteristics of several selected diseases from the group of diseases important for transfusion medicine in Croatia, basing on data of the Epidemiology service of the Croatian national institute of public health. Here, in the Abstract, some of them can be mentioned: HIV/AIDS continues to show rather favorable low incidence trend with very slow increase of total number of infected (prevalence), hepatitis B intensity is decreasing further, owing, among other, to systematic vaccination, hepatitis C trends are stationary, syphilis is rare and under control, but slightly increased numbers are observed in last two years, EBV infection i.e. infectious mononucleosis incidence oscillates at levels reached after decades of gradual increase, with a slightly marked upward trend. For a rational policy in donor selection and systematic blood testing, a constant insight in epidemiological situation in Croatia, Europe and in the World is important, using reliable sources, like Croatian National Institute of Public Health, European center for diseases control and prevention ECDC, and the WHO.

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Options in diagnostics of blood-borne infections: yesterday, today and tomorrow

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In the first 50 years of the 20th century many diagnostically useful tests were developed. Primarily they were focused on making the diagnoses and therefore applied after a physician had narrowed the differential diagnosis to a short list of possibilities. The main goal was diagnosis and treatment if possible. In the same period of time serology, as a laboratory discipline was established.

The battery of methods for antibody later on and antigen detection was developed like gel precipitation (immunodifusion, counterimmunoelectrophoresis), agglutination (direct, passive and inhibition), radioimmunoassay, enzymeimmunoassay (chemiluminiscence), flow cytometry etc. The sensitivity of various immunoassays differed, and with each new method increased, as well as specificity by the use of monoclonal antibodies and recombinant antigens. Test evaluation for given clinical field settings to ensure quality is become common laboratory practice.

In the 21st century, with automation and molecular probe techniques and bioinformatics, clinical laboratory has shifted from diagnosis to prognosis, risk stratification, treatment selection and patient monitoring. Rapid developments of molecular tests enabled studies of infectious diseases at the molecular level. Genomes of numerous pathogens and their vectors are sequenced helping scientist to understand how viruses enter the cell and replicate, if they react with the cell proteins, how pathogen evade immune defense and what is the mechanism of virulence. Next Generation of Sequencing (NGS) after the human genome mapping open the new era in laboratory medicine which is not only focused on pathogen characterization but also host oriented to identifying human genetic cause of susceptibility to particular infectious disease. Laboratories today are at the beginning of the huge transformation which include not

only methods, instruments, bioinformatics implementation but new generation of laboratory medicine specialist or multidisciplinary specialist's team.

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Molecular diagnostics of viral blood transmissible infections

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Nucleic acid amplification techniques (NAT) for viral testing have experienced a rapid development during the last 30 years and have been revolutionized the diagnosis of infectious diseases, particularly viral diseases. NAT has reduced the use of viral culture based methods and serological assays, because of faster turnaround time and significant shortening of window period. The introduction of fully automated platforms has allowed molecular diagnostic laboratories to report sensitive and accurate results. The goals obtained by NAT provide fast, highly sensitive, specific and accurate results which improve patients following and care. The use of molecular techniques such as real-time PCR or polymerase chain reaction with sequence specific oligonucleotide probes (PCR-SSOP) for virus detection, genotyping and quantification have high sensitivity, reproducibility and a broad dynamic range. A great number of qualitative and quantitative molecular virus assays, mostly based on RT-PCR technology have been described. Automation of these methods provides analysis of smaller volume of sample, ease performance and speed, low contamination risk, as well as better standardization.

The risk of transfusion-related infections dates back to the historical beginning of large scale transfusions in 1900s years. Because of potential transmission of virus during window period, NAT testing have implemented in many developed countries in blood screening. Monitoring the residual risk of systematically screened viruses, i.e. HIV, HCV and hepatitis B virus, is a priority for blood transfusion all over the world. Emerging/re-emerging agents are also the subject of active surveillance because some of them have been proven to be transmissible by blood, such as the West Nile, Chikungunya and Dengue viruses. Microarrays and advanced sequencing systems with highly sophisticated software's, contribute to the faster correlation between virus significant for transfusion medicine and the disease. This lecture will focus on the application of different molecular technology in the viral molecular diagnostic laboratory in transfusion service.

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Serological diagnostics of blood-borne infections

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Reported blood-borne infections (BBI) are due to prion, viral, bacterial, rickettsial, protozoan and nematode agents. Obligatory serology tests in Croatian blood donors testing are HBsAg (Hepatitis B Surface Antigen), anti-HCV (antibodies to Hepatitis C virus), HIV Ag/Ab (simultaneous detection of antigen and antibodies to Human Immunodeficiency Virus type 1 and/or 2) and anti-TP (antibodies to *Treponema pallidum*), and for organ donors also anti-CMV (cytomegalovirus), anti-EBV (Epstein-Barr virus), and anti-Toxo (*T. gondii)*. Clinical serology testing includes also hepatitis A and D, HTLV-I/II and *Borrelia burgdorferi*. Soon and hepatitis E testing.

Serological diagnostics of BBIs from the 1970s to the present has experienced tremendous progress in the development of new methods, new markers and highly sensitive tests. It has had 3 phases and each of these phases lasted approximately one decade, and led to the decreasing of post-transfusion infection risk as well as morbidity. Today serological diagnostics of BBI include several tests in combination. For example, highly sensitive and specific screening tests, and in the confirmation algorithms the combination of immunoas-

says (IA) and immunoblot (IB) tests. Confirmation of primary test reactivity of blood donors or patients includes for: a. HBsAg a combination of 2 IA tests (ELISA/CMIA and, if necessary, ELFA), the quantitative HBsAg and neutralization test, b. HCV a combination of 2 IA, anti-HCV and HCV Ag/Ab (ELISA/ELFA) and 2 different IB tests, and optionally HCV Ag, c. HIV a combination of 3 HIV Ag/Ab assays (ELFA/ELISA/CMIA) and anti-HIV, HIV-Ag and 2 different IB tests, d. Syphilis (anti-TP) - so-called reverse algorithm: a combination of highly sensitive total anti-TP tests and IgM antibodies (ELISA/CMIA) then non-treponemal test – RPR (Rapid Plasma Reagin), less sensitive treponemal test –TPHA (Treponema Pallidum Hemagglutination Assay) and 2 different IB tests. For other HBV markers, e.g. anti-HBc and anti-HBe, when the reactivity of these tests are outside the conventional serological profile (e.g. anti-HBc only positive or only anti-HBe positive) or is weak reactive, 3 different tests are used and then for the final result we have a rule "2 of 3". Confirmatory anti-HBc test would be of great help.

Serological diagnostics algorithms of BBI, mentioned above, with implemented ISO 15189, have great diagnostic and prognostic value (therapy monitoring, seroconversion or seroreversion, the success of vaccination) and give a fast and reliable results to the patients and their doctors.

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Liver transplantation in HBV and HCV infection – laboratory follow up

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Chronic HCV has emerged as the most common indication for liver transplantation in western world. In Croatia it represents the second most common indication (15% of all patients). HBV disease is less common (up to 5%).

Post-transplant disease recurrence occurs in nearly all patients with detectable pretransplant viremia, compromising the lifesaving significance of transplantation. Disease progression is accelerated in post-transplant patients. Of those with HCV disease recurrence, 10-30% develop cirrhosis within 5 years and have diminished survival rates of 41% and 10% at 1 and 3 years, respectively. The most detrimental pattern of HCV and HBV disease recurrence is fibrosing cholestatic hepatitis, occurring in 7-15% of recipients and leading to early graft failure, decompensation and death.

Follow up diagnostic procedures performed to transplanted patients are directed to recognition of signs of early and late posttransplant complications. Early period is mainly compromised with surgical complications, bacterial infections and acute cellular rejection. Later follow up period (after 6 months) is predominantly directed to recognition and treatment of recurrence of primary disease (including HCV and HBV hepatitis) and complications of immunosuppressive therapy.

Diagnostic methods related to diagnosis of HBV and HCV infection in transplant patients are not different than to overall population. However, overlap between laboratory findings in disease recurrence with other posttransplant complications brings close patient and viral infection follow up to highest importance. Interpretation of all other laboratory and morphological diagnostic findings includes assessment of molecular and serological markers of viral infection. Of great importance is close follow up of patient under antiviral treatment. Except from overall applicable treatment follow up rules in general population, it is of great importance to stress out the importance of regular HBV viremia and HBs antibody/antigen assessment while on antiHBs immunoglobulin prophylaxis.

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Analysis and results of serological testing of solid organ donors

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The success of transplantation and graft survival are closely related to the absence of infection of

recipient by infected graft, reactivated recipient's latent infections, nosocomial infections and community acquired. The exact risk for infection associated with organ transplantation is unknown but correlates with multiple factors, including epidemiology of specific infectious exposures, tissue tropism, and transmissibility of potential pathogens through transplantation and from the recipient side it depends on type of immunosuppression therapy and degree of achieved immunosuppression. The incidence of postransplant infections in the USA, the country with the highest number of transplanted organ (app. 30000 / year) is estimated at 1%. There are several reports of infections transmitted through transplanted organs. They include viruses: hepatitis B and C, herpes viruses, human T-cell lymphotropic viruses (HTLV) 1/2, West Nile virus, rabies, LCMV, polyomavirus BK/JC, HPV, parvovirus B19, HIV, bacteria: *mycobacteria*, *meningococcus*, *T.pallidum*, and parasites: *Plasmodium spp.*, *Babesia, Toxoplasma gondii, Trypanosoma cruzi*, *S stercoralis* and several types of fungi. Current organ safety rely on reviewing the donor's medical history and quality of blood transmissible disease mandatory testing. Organ donor testing includes serologic tests for HIV, HBV, HCV, CMV, EBV and nonviral agents *T. gondii* and *T. pallidum*, and more and more frequently also nucleic acid tests (NAT) for HIV, HCV and HBV. Guidelines are made to outline the appropriate blood collection and transportation, screening processes and results validation to minimize organ loss due to incorrect testing results. Croatian Institute of Transfusion Medicine (CITM) provides mandatory testing (serology and NAT) for the entire country. There was no confirmed cases of pathogen transmission by grafted solid organ due to testing failure. Prevalence of HIV, HBV, HCV and syphilis in solid organ donors is low and expected as it is high prevalence of ubiquitous pathogens like CMV, EBV.

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Characteristics of molecular testing of blood and organ donors

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Implementation of molecular testing, better known as nucleic acid testing (NAT), in screening of blood donors began at the end of the past century. It represents a routine screening test in most of the world's developed countries which greatly reduces risk of viral infection transmission by shortening a window period. NAT is performed for three viruses of great importance for transfusion medicine; hepatitis B, hepatitis C and human immunodeficiency virus (HBV, HCV, HIV). Some countries implemented also NAT for Parvo B 19 and hepatitis A and E virus due to plasma fractionation requirements. West Nile, Dengue and chikungunya virus are being tested with NAT in some endemic regions with emerging pathogens infection present. Depending on state regulation and viral infection prevalence, testing is performed in mini-pool NAT (MP-NAT) or NAT on individual donation (ID-NAT). Commercial NAT test for screening are based on multiplex real-time PCR or TMA methodology. They are fully automated and robust. Operators must be highly trained, following a strict rules regarding workflow and workspace to avoid cross-contamination and obtain high specificity and sensitivity of testing.

In organ transplantation it is of great importance to avoid the transmission of blood-borne viruses to the recipient. Although it is not mandatory in most of the countries, routine NAT testing of organ donor greatly reduces the risk of HBV, HCV and HIV transmission. When the organ donor is infected, by adding more molecular tests it is possible to establish valuable information on viral genotype and load. For NAT testing of heart- and non-heart beating organ donors, the quality of sample has to be obtained in order to avoid presence of inhibitory substances and false negative results. In March 2013. Croatia has started with routine NAT testing of voluntary blood donations, autologous donations and organ, tissue and steam cells donors.

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Quality assurance in accredited serology laboratory: a brief overview

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Quality assurance in accredited serology laboratory due to ISO 15189 is monitoring of entire process of diagnostics of infections which starts and ends with the patient. It should be used to identify procedural and technical problems, check the adequacy of current techniques and/or tests, establish the frequency of errors and to increase confidence in the procedures and reports given by the laboratory.

Quality assurance in Diagnostic virology and serology laboratory, is given by the UK Standards for Microbiology Investigations, Issued by the Standard Unit, Microbiology Services Division, Health Protection Agency. It includes: quality assessment (QA), quality control (QC), monitoring of laboratory equipment (MLE) and audits. But, to meet the requirements of ISO 15189 quality assurance should consist of well established: 1. QA - QA or proficiency testing usually includes External Quality Assurance (EQA): National External Quality Assessment Schemes (NEQAS) and/or External Quality Assessment Schemes (EQAS), and Internal Quality Assessment Scheme (IQAS), 2. QC - includes kit/test controls and internal QC controls (IQC); IQC samples should be included in all assays performed in the laboratory for validation of test kits and equipment, 3. equipment validation/qualification master plan – identify the processes or stages where equipment is involved; for each stage identify: failure mode, cause, effect and detection – risk assessment and after that schedule for equipment validation/qualification, 4. measurement uncertainty – define the performance requirements for accredited methods/tests; use possible measured quantity values, e.g. S/CO values and calculate CV and SD, 5. risk analysis – evaluate the impact of work processes on results, eliminate the identified risks and document decisions and actions taken, 6. quality indicators – for all laboratory phases, which should be periodically reviewed, 7. personnel certification – job description, continuos training program and evaluation, 8. audits.

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Assessment and risk management in the molecular diagnostic laboratory

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Risks that may occur during the daily routine work in molecular diagnostic (MD) laboratories are assembly and the possibility of harmful effects, factors and events that can lead to errors in the work and result with wrong lab reports. The risk assessment is a systematic quality control process of responsible experts or team. They could identify (define) risk, work on its analysis and evaluation, on control and monitoring, introduce the measures to reduce the perceived risk which provides better control of the whole working process in the MD laboratory. Responsible team should make an analysis of the whole working process to find the critical points and causes that contribute to the errors, which will reduce the risk and prevent wrong lab reports. Team should also take in consideration the current and potential non-compliance. Risks are divided according to the phases of the working process on the pre-analytical, analytical and post-analytical. The most numerous risks occur in the analytical phase can be categorized on the risks due to equipment failures or due to human error. Risk management in MD is the process of selection and control the safety procedures to achieve an acceptable level of safety which increases the probability of achieving the goal, timely and accurate lab report. The team responsible for the assessment and management of risk on the basis of their experience assesses and evaluates each risk as uncertainty in the work that can result in a deviation from the expected results. Risk management should be a comprehensive, systematic, continuous, proactive, flexible and forward-looking. This improves the working efficiency of employees, environmental protection and delivery of wrong lab report reduce to a minimum. The lecture will focus on examples of daily risks in MD laboratory which could be obtained and avoid if their origin and risk management is well defined and followed.

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