

Broketa M, Vince A, Draženović V, Sim R, Mlinarić-Galinović G. Non-radioactive digoxigenin DNA labeling and immunologic detection of HSV PCR products. J Clin Virol 2001;23:17-23.

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The aim of this study was: 1) to introduce non-radioactive labeling of herpes-simplex virus (HSV) DNA probe by digoxigenin-labeled dUTP; 2) to establish a rapid and reliable laboratory method for rapid HSV diagnostics; and 3) to compare the PCR method with the standard virology techniques, such as cell culture virus isolation and HSV direct fluorescent antibody test (DFA). The authors tested the efficiency of PCR method and non-radioactive labeling of HSV DNA probe for detection of HSV from 30 clinical specimens (skin and mucous membrane swabs). HSV was detected in the specimens by standard virology techniques and PCR. Replicated HSV DNA was non-radioactively labeled by random incorporation of digoxigenin-labeled deoxyuridine triphosphate (DIG-dUTP), and the hybrids were detected by the antibody conjugates and the appropriate enzyme-mediated staining reaction (DIG DNA labeling and detection kit non-radioactive, Boehringer Mannheim GmbH). HSV DNA was successfully multiplied and detected in the HSV-infected cell culture supernatant; however, it was not detected in the clinical specimen supernatant or sediment. HSV DNA was detected by direct PCR method in non-centrifuged clinical specimens. In conclusion, the PCR could be successfully used for diagnoses of HSV infections. Since the sensitivity of this method is partly limited by the virus quantity in the specimen, the authors recommend cultivating the virus in the cell culture at least 24 h prior to PCR. The use of non-radioactive labeling of hybridization DNA probes, such as random primed DNA labeling with digoxigenin-dUTP, has proven both sensitive and specific, and more appropriate for diagnostic purposes than radioactive DNA labeling to be used until standardized commercial tests appear.

Garaj-Vrhovac V, Želježić D. Cytogenetic monitoring of Croatian population occupationally exposed to a complex mixture of pesticides. Toxicology 2001;165:153-62.

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The paper describes a longitudinal study of possible genetic damage in Croatian workers occupationally exposed to a complex mixture of pesticides. The methods of choice were chromosomal aberration analysis, sister chromatid exchange analysis (SCE), micronucleus assay and comet assay. In order to determine primary genotoxic effects in workers, blood samples were taken after the workers spent 8 months in the production of pesticides. During the production all subjects were simultaneously exposed to a complex mixture of pesticides containing atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion. To detect DNA repair in lymphocytes of the same subjects the second series of blood samples was taken 8 months after the workers were removed from production. Regardless of the time sampling time the exposed workers showed an increased number of chromosomal aberrations, SCE frequency, micronucleus (MN) frequency, and values of comet assay parameters. After 8 months of non-exposure the workers showed a significantly decreased number of chromosomal aberrations, MN frequency, and DNA migration compared to the results of the first sampling, but it was still signifi-

cantly higher than in controls. Furthermore, the SCE frequency in the exposed subjects did not drop after the 8 months of non-exposure, which indicates long-term exposure to a mixture of pesticides.

Harjaček M, Ruperto N, Ostojić J, Bukovac LT. Paediatric Rheumatology International Trials Organisation. The Croatian version of the Childhood Health Assessment Questionnaire (CHAQ) and the Child Health Questionnaire (CHQ). Clin Exp Rheumatol 2001;19(Suppl 23):S40-4.

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The authors report the results of the cross-cultural adaptation and validation into the Croatian language of the parent's version of two health related quality of life instruments. The Childhood Health Assessment Questionnaire (CHAQ) is a disease specific health instrument that measures functional ability in daily living activities in children with juvenile idiopathic arthritis (JIA). The Child Health Questionnaire (CHQ) is a generic health instrument designed to capture the physical and psychosocial well-being of children independently from the underlying disease. The Croatian CHAQ-CHQ were fully validated with 3 forward and 3 backward translations. A total of 139 subjects were enrolled: 75 patients with JIA (19% systemic onset, 20% polyarticular onset, 17% extended oligoarticular subtype, and 44% persistent oligoarticular subtype) and 64 healthy children. CHAQ clinically discriminated between healthy subjects and JIA patients, with the polyarticular and extended oligoarticular subtypes having a higher degree of disability, pain, and a lower overall well-being when compared to their healthy peers. Also the CHQ clinically discriminated between healthy subjects and JIA patients, with the polyarticular onset and extended oligoarticular subtypes having a lower physical and psychosocial well-being when compared to their healthy peers. In conclusion, the Croatian version of the CHAQ-CHQ is a reliable, and valid tool for the functional, physical and psychosocial assessment of children with JIA.

Jakšić O, Vrhovac R, Kušec R, Kardum MM, Pandžić-Jakšić V, Kardum-Skelin I, et al. Clinical tumor cell distribution pattern is a prognostically relevant parameter in patients with B-cell chronic lymphocytic leukemia. Haematologica 2001;86:827-36.

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The authors introduce the model for tumor distribution (TD) assessment based on TTM scoring system, where TD value represents percentage of total tumor mass infiltrating peripheral blood and bone marrow (TD = TM1/TTM). TD in B-CLL can be categorized into 3 subgroups: pure leukemia if TD = 100%, predominantly leukemia if TD = 50-99% and predominantly lymphoma TD < 50%. Among 341 B-CLL patients there were 22.6%, 55.1%, 22.3%, pure leukemia, predominantly leukemia and predominantly lymphoma cases, respectively. TD parameter was strongly associated in univariate analysis with TTM size, Rai and Binet stages, spleen size and beta2-microglobulin. TD was associated with response to therapy and survival, with higher TD values translated into higher response rates and longer survival. However, in univariate and multivariate Cox analysis TD displayed much stronger relationship with prognosis in female patients, where it is the strongest independent predictor of survival along with age and Binet stage.

TD, a quantitative and simple clinical parameter, easily assessed in all patients, offers a reliable tool for evaluation of tumor cell distribution in B-CLL. It has independent and strong prognostic power in females, as opposed to males, possibly unmasking important, yet unrecognized, biological difference in B-CLL patients.

Klobučar H, Delimar D, Čičak N, Koržinek K. A secure locking knot for microsuturing. J Reconstr Microsurg 2001;17:331-4.

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Perfect balance between maximal suture strength and minimal foreign-body reactivity guarantees success, using microsurgical techniques. The proposed initial locking knot allows optimal edge approximation. It has strength, it is simple to master, and is not too bulky. The knot is kept in its first position, without danger of slipping, before securing it with an additional knot of two loops. No additional knots are necessary, because the completed knot may fail by breaking, but never by slippage.

Krhen I, Škerk V, Mareković Z, Davila NJ. Gentamicin concentrations in urine, cortex and medulla in an acutely obstructed kidney animal model. J Chemother 2001;13:389-94.

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Gentamicin was administered intraperitoneally, three times in 12 h to Hartley type guinea-pigs which had undergone complete unilateral ureteral obstruction with normal contralateral ureteral function for either 24 hours, 7 days or 21 days. Two hours after the last drug dose urine samples were collected from urinary bladder and obstructed ureter. Healthy and obstructed kidneys were then surgically removed from all sacrificed animals. Gentamicin concentration in urine of healthy kidney was 112-266 µg/ml, and in obstructed kidney 18-53 µg/ml, with a tendency of linear decrease over a 3-week obstruction period. The gentamicin concentration in obstructed renal cortex never exceeded one-third of the gentamicin concentration in unobstructed renal cortex. The maximum gentamicin concentration in obstructed renal medulla was 75% of the gentamicin concentration in unobstructed renal medulla.

Krželj V, Zlodre S, Terzić J, Meštrović M, Jakšić J, Pavlov N. Prevalence of G-6-pd deficiency in the Croatian adriatic coast population. Arch Med Res 2001;32:454-7.

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The objective of this study was to determine the prevalence of glucose-6-phosphate dehydrogenase (G-6-PD) deficiency among the population of the Croatian Adriatic Coast, part of the Mediterranean basin. The fluorescent spot test was used to screen 2,726 randomly selected high school students in the Croatian Adriatic coastal area. Fluorescence readings were performed at the beginning and at 3, 6, 10, and 25 min of incubation. Results were classified into the following three groups: bright fluorescence (BF), weak fluorescence (WF), and no fluorescence (NF). All NF and WF samples at 3 min were quantitatively measured using the spectrophotometric method. Twelve persons, 10 boys and 2 girls, were found to be deficient in G-6-PD, rendering a 0.44% prevalence of G-6-PD deficiency. All NF samples at fluorescent spot test were G-6-PD-deficient. WF at 3 min of the incubation period was present in 33 (1.2%) subjects, and only 2 (6%) were true positive. Fluorescence reading at 10 min of incubation omits five (41%) of the G-6-PD deficient samples. Prevalence of G-6-PD deficiency in the Croatian Adriatic coastal population is 0.44%. Fluorescent spot test for moderate enzyme deficiency is reliable in early fluorescence reading.

Pleština S, Gamulin S. Kidney ischaemia-reperfusion injury and polyribosome structure. Nephron 2001;89:201-7.

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Inhibition of protein synthesis and polyribosome disaggregation are the early events in cell injury provoked by various pathogenic mechanisms, including energy depletion. Polyribosome disaggregation might be expected to occur during ischaemia-reperfusion injury due to ischaemic energy depletion, but also due to detrimental effects of reactive oxygen species on various macromolecules and cellular structures. Mouse kidney ischaemia-reperfusion injury was provoked by temporary clamping of the renal artery. The polyribosome sedimentation pattern was analyzed by sucrose density centrifugation of kidney postmitochondrial supernatant. Ischaemia for 5 min in the mouse kidney provoked polyribosome disaggregation and an increase of monomer ribosome fraction which was augmented during 10-360 min of reperfusion. Recovery of polyribosome aggregates appeared between 6 and 24 h of reperfusion. Cycloheximide pretreatment prevented only polyribosome disaggregation caused by ischaemia and not that caused by reperfusion. This indicates different mechanisms of polyribosome disaggregation during ischaemia and reperfusion. It probably occurs in the former due to inhibition of initiation of translation, resulting in accumulation of unprogrammed monomer ribosomes, and in the latter due to the splitting of mRNA and breakdown of polyribosomes. Reperfusion did not increase ribonuclease activity in kidney cytosol, but increased the tissue concentration of malonaldehyde, indicating an augmentation in oxygen free radical generation. Possibly these may have caused a non-enzymatic breakdown of polyribosomes. However, pretreatment with allopurinol did not prevent polyribosome breakdown during ischaemia-reperfusion injury.

Sindić A, Aleksandrova A, Fields AP, Volinia S, Banfić H. Presence and activation of nuclear phosphoinositide 3-kinase C2beta during compensatory liver growth. J Biol Chem 2001;276:17754-61.

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Highly purified liver nuclei incorporated radiolabeled phosphate into phosphatidylinositol 4-phosphate (PtdIns(4)P), PtdIns(4,5)P(2), and PtdIns(3,4,5)P(3). When nuclei were depleted of their membrane, no radiolabeling of PtdIns(3,4,5)P(3) could be detected showing that within the intranuclear region there are no class I phosphoinositide 3-kinases (PI3Ks). In membrane-depleted nuclei harvested 20 h after partial hepatectomy, the incorporation of radiolabel into PtdIns(3)P was observed together with an increase in immunoprecipitable PI3K-C2beta activity, which is sensitive to wortmannin (10 nM) and shows strong preference for PtdIns over PtdIns(4)P as a substrate. On Western blots PI3K-C2beta revealed a single immunoreactive band of 180 kDa, whereas 20 h after partial hepatectomy gel shift of 18 kDa was noticed, suggesting that observed activation of enzyme is achieved by proteolysis. When intact membrane-depleted nuclei were subjected to short term (20 min) exposure to &agr;-calpain, similar gel shift together with an increase in PI3K-C2beta activity was observed, when compared with the nuclei harvested 20 h after partial hepatectomy. Moreover, the above-mentioned gel shift and increase in PI3K-C2beta activity could be prevented by the calpain inhibitor calpeptin. The data presented in this report show that, in the membrane-depleted nuclei during the compensatory liver growth, there is an increase in PtdIns(3)P formation as a result of PI3K-C2beta activation, which may be a calpain-mediated event.

Stavljenić Rukavina A, Topić RZ, Ferenčak G, Sučić M. A novel missense mutation C127R (FH Zagreb) in the LDL-receptor gene. Clin Chem Lab Med 2001;39:505-8.

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The authors employed the analysis of single-strand conformation polymorphisms to identify mutations in exon 4 of the low density lipoprotein receptor gene causing familial hypercholesterolemia. Three familial hypercholesterolemia heterozygotes had abnormal single-strand conformation polymorphism patterns. DNA sequencing revealed that the abnormal pattern of exon 4A was due to heterozygosity (T/C) at nucleotide 442. Nucleotide 442 is the first base of codon 127, and the T→C mutation (C127R) changes this codon from CysTGT to Arg CGT. Abnormal patterns of exon 4B were due to heterozygosity (A/G) at nucleotide 662: nucleotide 662 is the second base of codon 200, and the A→G mutation (D200G) changes this codon from AspGAC to GlyGGC. Mutation D200G was previously described as FH Padova, but mutation C127R (FH Zagreb) has not been reported previously. This novel mutation was confirmed by restriction endonuclease analysis with Dsa I. The screening of 420 familial hypercholesterolemia heterozygotes suggests that C127R and D200G account for about 0.7% of mutations causing familial hypercholesterolemia in Croatia.

Brozović A, Šimaga S, Osmak M. Induction of heat shock protein 70 in drug-resistant cells by anticancer drugs and hyperthermia. Neoplasma 2001;48:99-103.

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The altered constitutive and inducible levels of heat shock proteins 70 (Hsp70) in drug-resistant cells may influence the efficiency of combined hyperthermia and anticancer drug treat-

ment. In the present study, the constitutive levels of Hsp70 and induction of these proteins by hyperthermia and two anticancer drugs (used for resistance development) were determined in cervical and laryngeal carcinoma cells. The levels of Hsp70 were quantified by Western blot. Constitutive levels of Hsp70 were similar in parental and drug-resistant cells suggesting that Hsp70 is not involved in drug-resistance. Hyperthermic treatment induced Hsp70 in all examined cell lines but with different kinetics between drug-resistant and parental cells. Following the treatment with anticancer drugs, Hsp70 was induced only in cisplatin-resistant laryngeal cells. Kinetics of Hsp70 induction (stress-type and cell-type specific) was different in drug-resistant cells as compared to parental cells. The observed alterations in Hsp70 induction in drug resistant and parental cells should be taken into account when combined treatments (i. e. hyperthermia and anticancer drugs) are planned.

Vrdoljak E, Miše K, Sapunar D, Rozga A, Marušić M. Staging in untreated patients with small cell lung cancer. Neoplasma 2001;48:154-6.

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In order to describe the real biological behavior of the small-cell lung cancer we have analyzed survival rates of 66 patients with small-cell lung cancer who did not receive any specific anti cancer therapy. Also, objective of this study was to evaluate the staging system of the small-cell lung cancer. Untreated small-cell lung cancer patients with limited stage disease had statistically significant ($p < 0.05$) better survival rates in comparison to patients with extensive stage disease. T and N factor of the TNM classification did not influence the survival in untreated small-cell lung cancer patients. It appears that the TNM staging system is not predicting survival probabilities of untreated patients with small-cell lung cancer, while the two-stage system appeared very well based on survival probabilities of these patients.