J. Iran. Chem. Soc., Vol. 6, Suppl., November 2009, pp. S103-S114.

JOURNAL OF THE
Iranian Chemical Society

Modern Techniques in Biomedical Diagnosis

P-10-58-1

Gender determination in single bovine blastomeres by multiplex nested single cell polymerase chain reaction (MNSC-PCR)

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Preimplantation sex determination is a very applicable process at reproduction of valuable domestic animals for economical aims. The main objective of this study was to establish a rapid and reliable PCR-based method for the sexing of 8- to 16-cell stage bovine embryos. A sensitive and precise technique for the sexing of bovine embryos was developed using Multiplex Nested Polymerase Chain Reaction (MN-PCR) amplification of the amelogenin (AML) gene on the X- and Y-chromosomes and SRY gene on Y chromosome (male specific gene) of cattle. DNA sequence alignment between X-AML and Y-AML genes showed a 63bp length polymorphism in 5th exon. First round multiplex-PCR was designed for amplification of a conserved fragment of 146-bp from the SRY gene on Y chromosome and two fragments of 283-bp and 220-bp from the X-AML and Y-AML gene, respectively. In the next round, multiplex nested PCR was carried out to amplify a 121-bp fragment of SRY gene and a 233bp fragment of X-AML gene. PCR condition was optimized using fibroblasts with definite sex and number. The primers were successfully applied to bovine sexing from single blastomeres isolated from day-6 to day-7 cow embryos by direct MNSC-PCR. Very small copy numbers of DNA obtained from one or two cells in MNSC-PCR required some modification. More stringent condition was applied for the most sensitivity. The developmental potential from 8- to 16-cell stage embryos to the blastocyst stage was not significantly different for intact embryo, however this analyses is on going. In conclusion, we developed a rapid, effective and very precise MNSC-PCR method for the sexing of preimplanted bovine embryos using a single blastomere.

Keywords: gender determination, PCR, bovine, embryo, blastomere

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P-10-56-1

Haplotype diversity and linkage disequilibrium at the PAH gene in Isfahan population

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In the present study, haplotype and linkage disequilibrium (LD) estimation of the phenylalanine hydroxylase (PAH) gene was performed in a sample of Iranian healthy population. Three markers in the PAH gene region including BglII, EcoRI and VNTR were selected. Genomic DNA was extracted from 20 families (including both parents and one child) and 90 healthy unrelated individuals and genotyped using polymerase chain reaction followed by restriction fragment length polymorphism (PCR/RFLP). The haplotype frequency was estimated for 20 families by FBAT computer program. The estimation of LD was performed for 90 unrelated individuals with 2LD program. Nine different VNTR alleles were observed in Isfahan population which corresponded to 3, 5, 6, 7, 8, 9, 10, 11 and 13 core repeats. Analysis of the estimation of BglII- EcoRI- VNTR haplotype frequency using FBAT program showed that 10 haplotypes had the frequency more than 5%, which could be introduced as informative haplotypes in this population. Furthermore, the estimation of D' and $\chi 2$ using 2LD program showed absence of linkage disequilibrium between these three markers. This could indicate that a large percentage of the chromosomes in this population had rare or uncommon haplotype, which suggested that haplotype diversity was high and numerous haplotypes were observed in this population with the frequency lesser than 5%. These haplotypes could be suggested as the first informative haplotype for application in prenatal diagnosis and carrier detection of PKU families in Iranian population.

Keywords: phenylalanine hydroxylase (PAH), phenylketonuria (PKU), haplotype, linkage disequilibrium (LD), Iranian populatiuon

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P-10-70-1

Determination of hemoglobin concentration using halide ion-modified silver electrodes

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In this study, we have investigated the redox property of halide ions using silver electrode as a working electrode. As we can see, the cathodic and anodic peaks of: a) Chloride were in the range of -58 and 162 mV, b) Bromide in the range of -132 and +56 mV, c) iodide in the range of -400 and -188 mV, respectively. When hemoglobin concentration was increased in any solution, a peak change was seen for all of the halide. We saw a positive shift in cathodic and anodic peaks of I- and Br-, while an increase in current was observed for the cathodic and anodic peaks of Cl-. After that, we investigated that different concentrations of hemoglobin could be observed in Br solution. We also investigated different Br scan rates in the Br solution in the presence of 35 μM hemoglobin and increased current is related

to increased scan rate. Satisfactory results have also been obtained for the determination of hemoglobin in clinical blood samples.

Keywords: silver electrode, halide ions, hemoglobin determination

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P-10-134-1

Down-regulation of the effect of FSH in the presence of Müllerian Inhibitory Substance: A pioneering study of the in-vitro growth and maturation in Syrian mice preantral follicles and enclosed oocytes

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The aim of the present study was to investigate the inhibitory and combined effect of MIS and FSH on in vitro follicle development. To determine the effect of MIS on the follicle and oocyte growth, the preantral follicles (diameter, 95 ± 5 µm) were cultured in the TCM-199 medium alone (control) and in the presence of different concentrations (100, 200, 400, 600, 800, 1000 and 1200ng/ml) of MIS. In the presence of 800ng/ml MIS, the follicle diameter increased from 113 to 159 µm ($p<0.001$) while, the survival rate showed an opposite and negative effect of MIS on the survival rate (25%) as compared to the control (28%), where $p=0.042$ ($p>0.01$). While, oocyte maturation (23%) and GVBD rates (36%) increased significantly in the culture groups exposed to 800ng/ml MIS as compared to the controls with 2% maturation and 9% GVBD rate ($p<0.0001$). To determine the effect of MIS and FSH, preantral follicles were cultured with 1) 800ng/ml MIS, 2) 100mIU/ml FSH and 3) 100mIU/ml FSH + 800ng/ml MIS. Decreased follicle diameter (171µm) was seen in experiment 3) as compared 2) (190µm). Oocyte maturation (28%) and GVBD rate (39%) increased significantly in the culture groups exposed to 800ng/ml MIS + 100mIU/ml FSH as compared to the controls (3% maturation, 9% GVBD; $p<0.0001$). MIS inhibits the effect of FSH on the growth and maturation of preantral follicle and enclosed oocytes in the in-vitro cultures.

Keywords: MIS, MIS-deficient, differentiation, mice, FSH

P-10-134-2

Local extra- and intra-ovarian factors: Control of in-vitro growth and differentiation in mice follicles

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Regulation of ovarian activity is an integrated process encompassing both extra-ovarian signals and intra-ovarian factors. Ovarian functions result from the evolution of a morphological unit, the ovarian follicle, which consists of a central oocyte surrounded by granulosa cells and other layers of somatic theca cells. The maturation of the follicle proceeds through primordial, primary, and secondary stages of development and is controlled by various factors produced in the ovary. Locally produced growth factors, the members of the transforming growth factor-β super-family, work in concert with gonadotropins throughout the follicular growth and have significant effects on follicle selection. The complexity of the interrelation of the events controlling follicular growth and ultimate acquisition of developmental competence is under continuous investigation. It is generally believed that follicular atresia and luteolysis occur by mechanisms that accompany a highly organized type of cell death, called programmed cell death or apoptosis. The present review reports a variety of intra-ovarian factors, involved in the follicular development. Elucidation of the mechanisms that regulate follicular development may lead to the prevention of female reproductive disorders or other pathological conditions and to the development of new culture methods for in-vitro maturation (IVM) and in-vitro fertilization (IVF) of the mice follicles.

Keywords: mice, follicle, intra-ovarian factors, growth factors, in-vitro maturation

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P-10-93-2

Conjugated linoleic acid (CLA) production and lipase-catalyzed interesterification of purified CLA with canola oil

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In this study, two important isomers of CLA, i.e. c9,t11 and t10,c12, were produced up to approximately 73% of total fatty acids, employing alkali isomerization of safflower oil, followed by purification with only one-step urea crystallization to 85.6%, while the recovery of the purification process was 35%. Interesterification (acidolysis) of purified CLA with canola oil was then conducted by Thermomyces lanuginosus lipase. The CLA content incorporated into the triacylglycerols (TG) was 26.6 mol-percent after 48 h of reaction time. Physical and chemical properties of the TG were then changed according to the degree of substitution of oleic acid in canola oil with CLA.

Keywords: canola oil, conjugated linoleic acid, enzymatic interesterification, production and purification

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O-10-233-2

Aberrant methylation of HIC1 and RASSF1A genes and their association with MTHFD1 G1958A polymorphism and major clinical and pathological features of breast cancer

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Aberrant methylation of CpG islands acquired in breast tumor cell in promoter regions of tumor suppressor genes is associated with the silencing of their genes expression. Genetic factors that alter the DNA methylation levels in normal and tumor tissues could therefore influence the susceptibility to this tumor phenotype. In the present study we determined the frequency of aberrant methylation of HIC1 and RASSF1A gene promoter regions and their association with MTHFD1 G1958A polymorphism and major clinical and pathological features of breast cancer. DNA was extracted from 81 primary breast tumors belonging to Iranian woman patients. Gene promoter

methylation was analyzed by methylation- Specific PCR and a genotype analysis was performed by restriction length polymorphism. We detected HIC1 hypermethylation in 79% of invasive and metastasis tumors and RASSF1A gene hypermethylation in 50% of them. We found no association between HIC1 and RASSF1A genes hypermethylation and MTHFD1G1958A polymorphism. The analysis of methylation distribution indicates a statistically significant association between methylation of the HIC1 and RASSF1A gene promoter ($r=0.24$, $p=0.02$). There was a correlation between hypermethylation of HIC1 locus and nodal involvement in the studied population ($p=0.03$). We found a significant association between total methylation and nodal involvement ($p=0.01$) as well as tumor size 2-5 cm ($p=0.02$) in all cases. Our data indicate that coincidence methylation of HIC1 and RASSF1A CpG islands hypermethylation can be used as epigenetic markers to detect the malignant progression of human ductal and lobular breast carcinoma in Iranian women patients.

Keywords: breast cancer, HIC1, RASSF1A, CpG island methylatioin, MTHFD1 G1958A polymorphism

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P-10-233-3

New approach in quantification of HER2 and EGFR genes amplification by high-performance liquid chromatography

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Human epidermal growth factor receptors (HER2)/neu and EGFR are amplified in breast and ovarian cancers. The HER2 or EGFR status of a tumor may provide an indication of the response to HER2 or EGFR-targeted therapies. Therefore, their amplification should be assessed in each breast and ovarian tumors. Immunohistochemistry is the most commonly used method for HER2 and EGFR expression but its interpretation is confounded by the lack of non-standard techniques. The accurate methods of Real Time PCR (RTPCR) or fluorescence in situ hybridization (FISH) are rather expensive and complicated. The aim of this study was to perform high-performance liquid chromatography (HPLC) for accurate and rapid quantitative analysis of HER2 and EGFR oncogenes amplification. DNA was extracted from 30 primary breast tumors. A target gene and a reference gene are co-amplificated by duplex PCR. We developed the use of HPLC for separation and determination of the quantity of PCR products through an anion-exchange DEAE column in 15 minutes and in a single step. Optimum resolution was obtained by using NaCL gradient and a column temperature of 50°C. In order to confirming our results, the HER2 and EGFR genes amplification were analyzed for 10 tumor samples by RTPCR as golden standard. Also the results of HPLC amplification folds for HER2 were compared with IHC. Our results were confirmed by RTPCR and showed HPLC analysis is more accurate than IHC and cheaper than RTPCR or FISH for routine measuring HER2 or EGFR expression.

Keywords: HER2, EGFR, amplification, HPLC, breast cancer

P-10-93-5

Effect of oat bread consumption on blood sugar and blood cholesterol

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A great attention has been made by fortification of bread with oat diet fibers. Oat fibers are known as a lowering substance of blood sugar, cholesterol, triglyceride, HDL and LDL. In this study 25 percent of wheat flour was replaced with oat flour to produce oat wheat bread and to investigate the effect of oat bread consumption on blood sugar, cholesterol, triglyceride, HDL and LDL. The extraction rate of Roshan variety wheat flour used in this study was 78 percent. Oat flour was prepared by milling of Caliber variety oat. To facilitate dehulling oat kernels was preheated at 75ºC for 20 minutes. Dehulled kernels were heated at 100°C for 1 hour period to prevent enzyme activity and bitterness taste of oat flour. Baked oat bread was consumed by 21 volunteers with high blood sugar or high blood cholesterol. The amount of consumption was 150 grams bread per day with normal three daily meals during 15 days period. Blood samples were taken from volunteers before and 10 and 15 days after bread consumption, and fasting glucose, cholesterol, LDL and HDL of the samples were measured. The results showed that the fasting blood sugar, cholesterol, LDL and HDL of oat bread consumers significantly lower than the time before oat bread consumption. It showed also that triglyceride levels of consumers' blood samples were not different with those before bread consumption; statistically. 25 percent oat flour bread reduced fasting blood sugar, cholesterol, LDL and HDL.

Keywords: bread, blood sugar, cholesterol, HDL, LDL

P-10-299-3

Sterilization of steel surface by H2O2+Ag+ solution as a surface disinfectant

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Nowadays antibacterial reagents which act on bacteria without making resistance became important. But another important aspect is the lower toxicity properties. In a pilot study we experienced that a solution composed of hydrogen peroxide (H_2O_2) and silver ions (Ag^+) could reduce or block the viability and growth of three genuses of bacteria including E.coli, Klebsiella and Proteus. Then we hypothesized that this reagent may act as a good surface disinfectant. We investigated the effect of this solution on viability of aforementioned bacteria on the steel surface. Initially we sterilized ten steel surfaces using alcohol and fire, and then bacterial cultures on Eosin Methylene Blue (EMB) agar from sterilized surface confirmed that no bacteria existed and sterilization was well. In the second step we contaminated the steel surface with a heavy suspension of bacteria in PBS buffer and after 15 minutes the culture confirmed the surface was contaminated with bacteria. In the third step we contacted the $H_2O_2+Ag^+$ solution to the surface and after 15 minutes bacterial cultures were performed. After 24 hours incubation on 37° C there were a significant differences for bacterial growth on EMB agar between 3 above described steps by Wilcoxon analysis where $P=0.008$ for E.coli, $P=0.014$ for Klebsiella, $P=0.002$ for Proteus. Here we showed $H_2O_2+Ag^+$ solution in 30ppm

and 30ppb concentrations, respectively, maybe a power full surface disinfectant. Also the catalase enzymes of these bacterial may be necessary for a better function of aforementioned solution.

Keywords: bacterial growth, hydrogen peroxide, silver ion, surface disinfectant

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P-10-184-1

Comparison of multiplex PCR and O & H typing techniques for detection of enteropathogenic Escherichia coli (EPEC)

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Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children, especially in developing countries. Among the bacterial pathogens diarrheagenic *Escherichia coli* (DEC) is an important agent of endemic and epidemic diarrhea world wide. EPECs are defined as intimin containing diarrheagenic *E.coli* isolates. The detection of this group of diarrheagenic *E.coli* is based on serological and newly developed molecular methods. In this study 500 *E.coli* isolates from children with diarrhea were subjected to serological typing and a multiplex PCR. The PCR is based on *stx1*, *stx2*, *escv* and *bfp* genes and it divides strains in to two groups, typical and atypical EPEC. Serology of the isolates with O typing revealed that 37 strains were typable with O antisera and they belong to 10 different O serogroups (O111, O55, O86, O26, O127, O42, O119, O114, O142 and O126). The H typing was performed by PCR-RFLP method and 14 different H types were found among the isolates. On the other hand the multiplex PCR revealed that 41 isolates are EPEC of which 27 were found to be typical and 14 isolates were atypical. The results obtained here revealed that the multiplex PCR used in this study could be considered as suitable technique for detection of our EPEC isolates.

Keywords: EPEC, multiplex PCR, serotyping, intimin, diarrhea

P-10-93-6

Comparing the amount of alpha amylase production in native and mutant Bacillus Subtilis PTCC1525

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Alpha amylase is an extracellular enzyme produced by many microorganisms including bacillus species. This enzyme is extensively used in starch liquefaction, food, paper, sugar and pharmaceutical industries. In the present study we compared the amount of alpha amylase production by mutant B. subtilis and the parent strain. The mutant strain of B. subtilis was achieved by mutagenesis (using EMS, ethyl methane sulfonate) and vast screening production. Then the best mutant strain with highest production was selected. Starch hydrolysis tests were carried out by adding iodine solution. In comparison to the wild type, production of alpha amylase was 2-folds better. The expressed enzyme was observed as clearing zones on agar plates or in zymogram. Enzyme synthesis occurred at temperatures between 25 and 45°C with an optimum of 30°C. It was observed that mutant B. subtilis has a maximum activity at pH 6 while the native form has utmost activity at pH 6.5. The optimum pH for amylase production and assay were the same. Analyses of the enzyme by SDS-PAGE revealed a single band with molecular mass about 50 KDa. So our result showed that the mutant alpha amylase may be suitable for high production in industries such as liquefaction of starch, in detergent and textile and in other industrial applications.

Keywords: alpha amylase, mutagenesis, starch hydrolysis, zymogram

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P-10-457-2

Telomerase targeting is way to cancer therapy

R. Arezumand

Telomerase is a ribonucleoprotein enzyme with two major subunits: One is an RNA that template to synthesize telomeric repeat in end of chromosome (hTR: human telomerase RNA) and other subunit is a catalytic subunit with reverse transcriptase activity that synthesize telomeric repeat with templeting hTR that called (hTERT: human telomerase reverse transcriptase). Telomerase has activity in stem cells, germ cells and immortal cells. Activity of telomerase in cancerous cells is necessary to immortality cancer cells. Apprizing of telomerase activity and its expression in cancers is detectable with telomeras repeat amplification protocol (TRAP assay) and Quantitative PCR. Furthermore Targeting hTR and hTERT and the other subunit of telomerase or telomere (e.g telomere association protein, hetro duplex DNA and RNA …) could due to dysfunction or non function in activity of telomerase and in resulted inhibitory of cancer. Telomerase targeting done by nucleoside and non nucleoside analog, interference RNA, antisense RNA, G-quaderplaxe agent, inhibition of phosphorylation of hTERT, inhibition of transcription of hTERT and etc. Furthermore association of telomerase targeting with the other therapies such as chemotherapy or radiation in decreases of drug resistance or increases the effect of therapeutic were very efficient. With this descriptions detection of telomerase activity and inhibitory of its activity could used in diagnostic and prognostic of cancers and in cancer therapy.

Keywords: telomerase, cancer, hTR, hTERT

P-10-58-2

Effect of lysis strategy in accuracy and repeatability of sex determination by single cell Polymerase Chain Reaction method

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Sex determination of early stage mammalian embryos has important implications for livestock management and breeding. Single-cell PCR provides a valuable tool for genetic characterization with a limited amount of starting material. The main objective of this study was to evaluating of different methods applicable to the single cell DNA preparation for embryos sex determination in the early stages. In this study, we have introduced an efficient protocol for DNA extraction step in the single cell PCR method. We compared three different lysis methods including; one physical (Nitrogen Freezing & thaw) and two chemicals (potassium hydroxide/dithiothreitol; KOH/DTT lysis protocol) and (Nonidet P40/Proteinase K protocol) for utilizing in the single cell PCR procedure. The results of multiplex nested single cell PCR using NP40/PK protocol was eminently more repeatable and efficient for routine applications in the laboratory in compared with the other

protocols analyzed in this study. Our results showed that the efficiency of amplification of the target genes in the single blastomer PCR was 94% with NP40/PK protocol, 88% for KOH/DTT lysis protocol and 64% by using of Freeze and thaw protocol. We concluded that the NP40/PK protocol was the most efficient method for extracting DNA from a single cell and could be particularly useful for sexing of bovine embryos in the early stages.

Keywords: single cell-PCR, cell lysis, sex determination

O-11-534-1

First report on the shortest CPB peptide chain in the Leishmania donovani complex & bioinformatical interpretations in relation with this mutation

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Visceral Leishmaniasis (VL) or Kala-azar is an important infectious disease in northwestern Iran. Members of the L.donovani complex, Leishmania donovani and L.infantum, are the two main parasites causing the disease in the world. Identification and determination Leishmania parasites characters causing VL in human is necessary to improve our understanding about epidemiology of the disease. A part of CPB gene of the Leishmania parasites isolated from sandflies was PCR amplified and sequenced for strain typing and molecular characterizations. Three dimensional (3D) protein structure, N-glycosylation, and T-cell and B-Cell epitopes were tested in the AA peptide using bioinformatics tools. For the first time, we report that both agents of kala-azar L. donovani and L. infantum are circulating by sand flies of Phlbtomus perfiliewi transcaucasicus in North West Iran. DNA analysis of the CPB gene showed a cytosine insertion at 5′ end of the proofreading frame of the gene resulted in a stop codon (TGA) seven AA further down and hence translation is halted. This caused a short amino acid chain with only 76 AA much shorter than normal CPB peptide with 234-247 AA. This mutation has not been found in the L.infantum strain resulted in a normal CPB peptide. AA analysis showed no N-glycosylation site, T-cell and B-Cell epitopes on the short peptide of L.donovani strain. This is the shortest CPB peptide chain reported for the Leishmania donovani complex in the literature. This short peptide could have an effect on host-parasite and vector-parasite interactions. Since the CPBs genes have important implication on host–parasite and play key roles in infection and expression of the disease, further studies on the L.donovani parasite and its diminutive peptide necessitate to improve our understanding about the epidemiology of VL disease in Iran.

Keywords: CPBs, host–parasite interactions, Kala-azar, Iran, Leishmania donovani

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P-10-534-2

Discrimination between main causing Visceral Leishmaniasis, L. infantum and L. donovani using two molecular assays: Species-specific PCR and PCR-RFLP

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Discrimination of Leishmania infantum and L.donovani, the members of the L. donovani complex, are important for diagnosis and epidemiological studies of visceral leishmaniasis (VL). We have developed two molecular tools including a restriction fragment length polymorphisms of amplified DNA (PCR-RFLP) and a specific-species PCR that are capable to discriminate L.donovani from L.infantum. Typing of the complex was performed by a simple PCR of cysteine protease B (cpb) gene followed by digestion with DraIII. The enzyme cuts the 741-bp amplicon of L.donovani into 400 and 341 bp fragments whereas the 702 bp of L.infantum remains intact. The designed primer is specific for L.donovani and is capable of amplifying a 317 bp of 3' end of cpb gene of L.donovani whereas does not generate amplification for L.infantum. The species-specific primer and the restriction enzyme were designed based on 39 bp insertion/deletion (indel) occurred in the middle of cpb gene. Both assays could differentiate correctly the two species and are reliable and high-throughput alternatives for molecular diagnosis and epidemiological studies of VL in various foci.

Keywords: Leishmania donovani, Leishmania infantum, PCR, PCR-RFLP, species identification, Visceral Leishmaniasis

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P-10-311-1

Synthesis of Poly-HRP dextran complex for high sensitive detection technology

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Recently modern diagnostic tests are based on the well-known ELISA format. Such assays, found to be first and foremost safer and easier than the early radioimmunoassay. According to this verity, several hydrogen peroxide detection methods have been designed. An increase in the sensitivity of immunoassays has always been considered as one of the major trends in development of this technology. Synthesis of polyHRP-Dextran-Antibody is a step toward achieving this trend. To provide this complex, Dextran-Aldehyde was prepared according to oxidation with sodium periodate. Activated polymer was then coupled to lysine amino acids and the process was checked with TNBSA and Kjeldahl methods. HRP was conjugated to free amino group of lysine and the stage's accuracy was showed by SDS-PAGE electrophoresis. Finally, the results confirmed the complete synthesis of poly-HRP Dextran complex by this procedure. So, after conjugating the complex to antibody, it can be applicable in high sensitive detection technologies. Attaching the complex to any antibody with biotin-streptavidin system and preparing direct Poly-HRP labeled antibodies will be a valuable approach to get punctual increase

of sensitivity in detection pathways that are used in ELISA, Immunocytochemistry and Immunohistochemistry methods.

Keywords: dextran, ELISA, immunoassay, horseradish peroxidase

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O-10-243-1

Hepatitis C virus Genotypes determination and application in routine clinical pathway in West Azerbaijan, Iran

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Hepatitis C Virus (HCV) has contaminated approximately 170 millions people all over the world. The isolated HCV genotype is a good predictor of antiviral therapeutic response and disease prognosis. Therefore in this study it was tried by using real-time PCR and melting curve analysis method, the genotype of affected patients with HCV to be determined. Using PCR and Elisa methods, serum sample of 310 suspicious affected patients with HCV were screened. Applying QIAamp Viral RNA Mini-kit method viral RNA were collected from serum samples of 160 positive patients. Using one-step RT-PCR protocol, genotypes of found HCV specified and determined according to the temperature melting (Tm) ranges. The frequency of 5 detected genotype of HCV in 160 cases (1a/b, 2a/c, 4, 2b and 3a) were as follows:77 (48.12%) were genotype 3a; 35(21.87%) genotype 2b; 19 (11.87%) genotype 2a/c; 16 (10 %) genotype 4; 13 (8.12%) genotype 1a/b.The most common HCV genotype in west Azerbaijan province in the northwest of Iran, is type 3a. Considering the obtained results it was possible to conclude that used PCR method is applicable in routine practice for HCV genotyping purpose.

Keywords: Hepatitis C, real-time PCR, HCV genotype

P-11-729-1

Evaluation for water stress tolerance in wheat genotypes at seedling stage through polyethylene glycol (PEG-6000)

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Polyethylene glycol of high molecular weight (PEG-6000) has been found to be most beneficial in creating osmotic stress in plants at seedling stage. To determine the effects of PEG-6000, twenty-one newly evolved wheat genotypes developed through conventional and mutation breeding techniques and four commercial drought tolerant check varieties Sarsabz, Thori (awn less), Margalla-99 and Chakwal-86 were screened at seedling stage. Different concentrations of PEG-6000 (0.5, 0.75 and 1.0 MPa) were used in comparison with control (0.0 MPa PEG). The parameters observed were germination (%), root and shoot length. Significant reduction in various traits was observed at high osmotic stress induced by high concentration of PEG-6000. At high osmotic stress (1.0 MPa), most of the genotypes germinated but couldn't produce roots and shoots. Genotypes NIA-8/7, NIA-10/8, NIA-25/5, ESW-9525, SI-91196, MSH-14, MSH-36, BWS-78, Sarsabz and Chakwal-86 showed significantly less reduction in germination percent at severe osmotic stress (0.75 MPa); indicated more tolerance to water stress at seedling stage. Eleven genotypes showed less reduction in root length at 0.5 MPa while fourteen genotypes have shown tolerance to severe stress (0.75 MPa). Genotypes NIA-8/7, ESW-9525, MSH-14, BWQ-4, BWS-77, BWS-78 and Sarsabz showed less reduction in shoot length at various osmotic stresses, which suggested tolerance of these genotypes to osmotic stress at seedling stage.

Keywords: wheat, water stress, polyethylene glycol

O-10-395-1

Purification of tetanus toxoid for application in DTP vaccine

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Tetanus is a neurological complication which is marked by the increase of muscular tonicity and spasm as a result of tetanospasmin. This protein is a strong toxin with a molecular mass of 150 kDa which is produced by clostridium tetani bacterium. Tetanospasmin consists of a single polypeptide chain and is released following an autolysis that forms a hetero-dimmer which in itself consists of two chains. The heavy chain with a molecular weight of 100 kDa intermediates the transfer to the receiver of the nerve cell and entering it, and the light chain (50 kDa) harnesses the neuron-transmitters. Today, vaccination against tetanus has been emphasized by the World Health Organization (WHO) as a necessity. Routinely, the toxoid used in preparation of the vaccine is taken from raw toxin by detoxification of toxin by formaldehyde, concentration of toxin by ultra-filtration, fractionation of the toxoid by salting out, desalination through dialysis and gel filtration G25. Although the mentioned procedures would result in the relative purity of this toxoid, other purification methods would be necessary for optimization of the purification methods. In the present study, after isolation and detoxification of toxin, for further purification, ion exchange chromatography DEAE-sepharose and gel filtration G100 were used. In this method tetanus toxoid with a purity of 3482 lf/mg PN was produced. The results of electrophoresis SDS-PAGE reveals the existence of the homogenous tetanus toxoid protein in the final product which can be used for preparation of a better vaccine as well as an anti-toxin with a higher quality.

Keywords: DTP vaccine, gel filtration chromatography, Ion exchange chromatography, tetanus toxoid

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P-10-683-1

Studying the hormone-receptor binding potency by the Immuno-Receptor Assay method

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Many different methods such as cell proliferation assay, Radio-Receptor Assay (RRA) and bioassay were used for evaluation of the biological activity and receptor binding potential of recombinant proteins, hormones and cytokines. Long time table course and application of radioactive hormones are disadvantages and limitations of these methods. Research for other useful methods for bioassay of recombinant

proteins and hormones are under study. The aim of this research was to study the application and effectiveness of Immuno-Receptor Assay (IRA) for the receptor binding potential of hormones in vitro. For this purpose recombinant human Growth Hormone (rhGH) and growth hormone receptors from rabbit hepatocyte microsomes were used as the model protein. First ELISA micro-titer plates were coated by partially purified growth hormone receptors. Then rhGH was interacted with coated receptors. Finally the fraction of bound complex was determined by classic ELISA detection system by using colorimetric substrates. Our result indicated that, the profile of hormone-receptor binding depended on hormone concentration and dose. The profile of IRA hormone-receptor diagram is comparable with RRA ones.

Keywords: ELISA, rabbit hepatocyte microsomes, rhGH

P-10-766-1

Partial purification and characterization of an extracellular haloalkaline protease produced by the moderately halophilic bacterium, Hallobacillus sp.AB9

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Among several strains of moderately halophilic bacteria that were isolated from various areas in Iran, one strain was selected as higher producer of extracellular protease and was used for further studies. Phenotypic classification and 16S rRNA sequence analysis placed this strain in the Halobacillus genus. This extracellular protease was secreted by a moderately halophilic bacteria and it seems to be a moderately haloalkalophilic and termophilic enzyme. This protease was partially purified by a successive combination of ammonium sulfate precipitation, Q-Sepharose ion exchange and Sephacryl S-200 gel filtration chromatography. The optimum conditions for cell growth and protease production were incubation in medium (pH 7.5) at 37°C and agitation speed of 220rpm for 48h. The partially purified protease was active between 30-70°C and lost more than 20% of its activity around 70°C. It was active over a broad range of pH 6.0-11.0 with the optimum at 7.5-10.0.

Keywords: protease, purification, halophile

P-10-782-1

Designing a new technique called CMA (Chimeric Primer-Mediated Amplification) for DNA amplification in isothermal condition

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Nucleic acid amplification methods play an important role in molecular biology. PCR is the most popular technique in this area. Isothermal amplification systems are the new approaches for amplification of DNA and RNA, easier and faster than PCR. In this study, we have designed and developed a new technique, called CMA (Chimeric Primer-Mediated Amplification), which is based on isothermal amplification. In this method, two enzymes are used; Bst Large fragment DNA polymerase and RNase H. The primers are Chimeric, consisting of DNA and RNA. A chimeric primer is made of three parts; a 4-5 bp portion of DNA in 5' end, followed by a 4-5 bp RNA portion and a 17-20 bp DNA in 3' end. After denaturation, the forward primer attaches to the single stranded target DNA, forming a hybrid of RNA/DNA in the middle of the primer. The RNase H enzyme distinguishes this hybrid and cleaves the RNA strand at different sites. This generates some free 3' OH ends, which are the substrate for DNA polymerase. The DNA polymerase displaces the single stranded DNA by its strand displacement activity, while it faces the double stranded DNA. The 3' end of reverse primer contains DNA which is complimentary to a part of displaced DNA stand and finally, a double stranded DNA is formed. This technique is able to amplify the target DNA in less than an hour. The temperature of the reaction is constant and between 60-65°C. It can amplify a target DNA from genomic DNA. Being simple to perform, specificity and sensitivity are other aspects of this technique.

Keywords: isothermal, method, chimeric primer, amplification

P-10-472-1

Fibrin fibers as a biodegradable scaffold in peripheral nerve repair

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Fbrin is the production of fibrinogen cleavage within the wound which cross-links with fibronectin during clot formation. The objective of the present study was to investigate the effect of using fibrin as scaffold in repairing cut sciatic nerve in rats. Twenty male wistar rats weighing 200-250g were randomly divided into two groups, autograft (A) and the fibrin scaffold (FS). The 5mm gap in cut right sciatic nerves was filled with autograft and FS in respective groups. Animals were then assessed by sensory, motor and EMG tests one, three, and five weeks following the induced injury. Results were then compared in both groups using unpaired t test. The trends of recovery were similar and comparable in both groups when the data obtained in the 3rd and the 5th week was crossed with those gained during the end of the first week post-transplant. The mean functional index of the sciatic nerve was in parallel in both groups throughout the five weeks of follow-up period. The mean motor response delay had a similar trend and was not significantly different in any group. So was the amplitude of the mean motor action potential. The data presented are in favor of the notion that the fibrin scaffold could potentially be looked upon as an alternative approach for autograft transplants in peripheral nerve injury.

Keywords: nerve repair, autograft, fibrin scaffold

P-10-794-1

The role of methylation in 5′ UTR CpG islands of BAX and Bcl2 in the endometrium of patients with endometriosis

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DNA methylation in Cpg Islands of higher eukaryotes has important roles in regulation of gene expression. Endometriosis is a medical condition in women in which endometrial cells are deposited in areas

outside the uterine cavity and it is a common finding in women with infertility. Apoptosis has been shown to be a pivotal regulator of endometrial function during the menstrual cycle and implantation. In this study, we investigated the role of aberrant DNA methylation in Bax gene promoter and Bcl2 gene promoter, which are important apoptotic and antiapoptotic proteins, in women with endometriosis. The endometrial tissues were collected from 10 patients with endometriosis and 10 normal women. Methylation status in 5′ regions of these genes was determined by methylation specific PCR (MSP-PCR). 60% of women with endometriosis showed methylation in bax 5′UTR compared to 40% in normal fertile women. MSP analysis of 5′UTR of Bcl2 showed that 50% of women with endometriosis had methylated allele compared to 70% in control normal women. We have shown that the pro-apoptotic gene Bax is methylated in women with endometriosis and apoptotic gene Bcl2 is hypomethylated in women with endometriosis compared to normal women (methylated Bax/methylated Bcl2=2). This research is in favor of importance of epigenetics modifications in women infertility.

Keywords: MSP, endometriosis, Bax, Bcl2

P-10-636-1

Molecular investigation of int-2 gene amplification in Iranian patients with ovarian cancer

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Ovarian cancer is the fifth most common gynecological tumor type worldwide. Asymptomatic clinical features and the lack of suitable screening tools account for its high mortality rate. One of the major challenges in cancer research aims to identification of oncogenic alternations because of potential diagnostic and therapeutic implications. To perform molecular analysis, paraffin-embedded samples of cancerous and non-cancerous tissues obtained from 30 Iranian women with ovarian cancer were screened for possible amplification of int-2 oncogene (on chromosome 11q13) which encodes a member of fibroblast growth factor family termed FGF3. Sequences from the int-2 oncogene and from a single copy reference gene interferon-gamma were amplified simultaneously by differential quantitative polymerase chain reaction (dqPCR). Recently developed software termed UVitec was used to compare the digital data obtained from gel documentation system. 11 tumors with increased int-2 copy number were identified which showed a 2.99 –fold increase compared to DNA from normal tissue of the patients and a 1.52 –fold increase compared to normal DNA of control individuals. Interestingly, 5 cases showed deletion at int-2 region, offering deficiency at the site of polymerization. The results of this study emphasize that women with positive familial history at puberty ages could benefit from screening for alternation at int-2 gene through peripheral blood testing in hope that this may lead to a marked increase in survived among patients diagnosed and treatment at primary stages.

Keywords: dqPCR, gene amplification, Int-2/FGF3, oncogene, ovarian cancer

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P-10-713-2

Acetic acid as an anabolic stimulator in xanthomonas campestris b82

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Xanthan gum, a microbial biopolymer produced by the Xanthomonas campestris, has many applications in food, agro-chemistry, pharmaceutical, and chemical and cosmetic industries. Xanthan is known as being biocompatible compound allowing its use in various medical applications such as implantation and or controlled release devices. Moreover, xanthan is considered as a biodegradable as well as bioadhesive compound. It promotes wound-healing effects. In medical and pharmaceutical applications, xanthan is also used as a component in hydrogels. Here, we studied the effect of acetic acids on xanthan production. It was found that the addition of acetic acid in to culture broth in late exponential growth phase increased xanthan production. In this study we evaluated energy balance of bacterial cells during xanthan production. For this purpose, ATP content of bacterial cells was measured after exponential phase of bacterial growth. We estimated whole ATP of bacterial cells during growth by reaction between luciferin and luciferase. The results indicated that in sublethal concentrations of acetic acid the amount of ATP in cells of Xanthomonas campestris decreased and concomitantly xanthan production increased. In mid-phase of the fermentation process, ATP concentration in the cells of Xanthomonas campestris was calculated after addition of acetic acid in different concentrations. ATP concentration was measured using a luminometer apparatus. Various counts (45300, 34000, 22600 RLU/s) and gum production (9, 10, 12gr/l) resulted from different concentrations of the acid (6.25, 3.12, 1.56 mM).

Keywords: ATP concentration, Xanthomonas campestris b82, acetic acid

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P-10-842-1

Production, purification and conjugation of murine anti-human CD133 monoclonal antibody

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CD133 antigen is selectively expressed on CD34bright haematopoietic stem and progenitor cells derived from bone marrow and human fetal liver and, blood. Due to its expression by haematopoietic progenitors, interest has been directed towards the potential of CD133 as a cell surface marker of haematopoietic stem cells. Regarding the significant role of CD133 in selection and characterization of haematopoietic stem cells for further applications, and concerning the complication of providing this valuable tool from other countries, we decided to produce this agent in our own laboratory. The mAb was produced through inoculation of hybridoma clone into peritoneal cavity of pristine-primed BALB/c mice and purified by protein G affinity chromatography. Purity was assessed on SDS-PAGE. Antibody was screened via ELISA and Flow cytometry and finally conjugated to a fluorochrome. Purified anti-human CD133 mAb exhibited positive reactivity with mobilized peripheral blood stem cells (mobilized PBSCs). Despite its reactivity with mobilized PBSCs, the antibody failed to stain a CD34+ cell line (KG1a) and also other peripheral blood mononuclear

cells. The positive data from ELISA and Flow cytometry analysis of the reaction among our purified mAb and mobilized PBSCs, and lack of interaction of this antibody with KG1a cell line and other peripheral blood cells, reveal its specificity for CD133 haematopoietic stem and progenitor cells , therefore the present mAb provides an alternative to CD34 for the selection and characterization of cells necessary for both short- and long-term engraftment, in transplant situations, for studies of ex vivo expansion strategies, and for gene therapy.

Keywords: CD133 antigen, haematopoietic stem and progenitor cells, immunophenotyping, monoclonal antibody

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P-10-411-1

ECD-HPLC method with glassy carbon electrode detector for analysis of nitric oxide (NO) produced by iNOS

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A large number of evidences indicate that nitric oxide (NO°), a pleitropic radical produced by a variety of cells, mediates the ability of macrophages to kill or inhibit the growth of tumor cells, bacteria, fungi and parasites. NO also regulates a broad spectrum of physiological functions such as vascular tone, neuronal transmission and immune defense. Macrophage inducible isoform of nitric oxide synthase (mac-NOS), like other isoforms catalyzes conversion of L-Arginine to L-Citrulline and nitric oxide via an oxygenase mechanism. A rapid isocratic method of high performance liquid chromatography system (HPLC) with a glassy carbon working electrode of electrochemical detector is set up for quantitative detection of trace amount of nitrite ion ($NO2^{-}$) in aqueous protein containing cell lysate, cell media, plasma, serum, urine and other body fluids. The solid extraction reversed phase cartridges (Sep-pack) are used for deproteinizing and purification of the samples. Nitrite ion is the only stable end product of autoxidation of nitric oxide (NO) ; which is a highly reactive paramagnetic molecule produced via the enzymatic conversion of L-arginine to L-citroline. The enzyme involved in this process is the inducible nitric oxide synthase (iNOS), the main isoform of the enzymes in macrophage and macrophage like cell lines such as Raw-264, J774, and Ic-21. Nitrite ion ($NO2^{-}$) in nanomolar concentration range is measured by the ECD detector with an amperometric cell, applied voltage of + 800 mV and Ag-AgCl as the reference electrode. Elusion buffer is 8 mM ammonium chloride containing 25% methanol, flow rate of 1 ml/min and column temperature set at 20° C. The reproducibility of sample preparation and analysis had a coefficient of variance (c.v.) less than 10 % in the cell lysates and cell media of the IC-21 cell lines. Therefore, this will be a reliable analytical method for the nitrite ion analysis under various conditions of cytokines, LPS, irradiation, or other chemical applications for evaluation of the probable over expression of the inducible nitric oxide synthase (iNOS) gene in these types of cells.

Keywords: ECD-HPLC method, inducible nitric oxide synthase (iNOS), IC-21 macrophage, glassy carbon electrode detector

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P-10-648-1

Crocin bleaching assay using Iranian saffron

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Crocin bleaching assay (CBA), is a new method for determination of antioxidant capacity. In CBA, abstraction and/or addition of hydrogen to the polyene structure of crocin results in the reduction of crocin's absorbance at 440 nm, which is considered as a value for antioxidant potential. Here CBA method was set up using crocin extracted and purified from Iranian saffron. Then the antioxidant activity of some known antioxidants, e.g., ascorbic acid, bilirubin, Trolox, some plasma samples from infants, and etc. were tested and compared with the standard methods The procedure was done in 2 different aquatic conditions, in the presence of >50% or >90% water. Results were analyzed by calculating of both "Krel" and "percent inhibition of crocin bleaching" (%Inh); Where $\%Inh = [(\Delta A0-\Delta A)/\Delta A0)]*100$, when $[AH]/[crocin]=1$. Our results indicated the following order for antioxidant potential of the above mentioned agents: Ascorbic acid>uric acid>Trolox. However, these results are very similar with the data reported by others, but they are related severely to the aqueous condition. In addition uric acid indicated different properties at different concentrations, so that it showed the antioxidant activity at low concentrations but at higher concentrations it acted as prooxidant. Bilirubin interfered with this test, possibly because its maximum absorbance is near the crocin. But the obtained data for the antioxidant capacity of the serums without it was comparable with FRAP assay. In conclusion, it seems that CBA as a simple method is very useful for determination of antioxidant potential of aqueous samples.

Keywords: Crocin Bleaching Assay, Iranian saffron, crocin, antioxidants

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O-10-903-1

Combination suicide gene therapy using E. coli nitroreductase and p53 therapy by adenovirus vector

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E.coli nitroreductase together with CB1954 is one of the enzyme/prodrug systems that can be applied for directed enzyme/ prodrug viral gene therapy. This enzyme is able to convert the prodrug CB1954 to a cytotoxic derivative that is able to kill tumor cells. Activated CB1954 leads to the formation of interstrand DNA cross-links and apoptosis starts in dividing and non dividing cells. At present, developed usage of p53 therapy in cancer and recombinant human adenoviral p53 injection (Gendicine) is the first approved gene therapy product. In this study, we investigated the effects of the p53 therapy in combination with the suicide gene therapy, NTR/CB 1954, on different cancer cell lines. We constructed a recombinant adenovirus carrying wild-type E.coli nitroreductase and human wild-type p53. This

consisted of the CMV immediate early promotor driving expression of NTR and p53. In 1μM and 10μM concentration of CB1954, IC50 of adenovirus encoding NTR was 100 & 1000 VP/cell (virus particle per cell), respectively. By using adenovirus encoding wild-type p53, IC50 was 300 VP/cell. Results demonstrated that combination of suicide gene therapy using NTR/CB1954, and p53 therapy gives synergistically decreased IC50 compared either modality alone. Therefore, the combination of NTR/CB1954 and p53 can significantly improve cancer gene therapy.

Keywords: cancer gene therapy, E. coli nitroreductase, CB1954, p53, adenovirus vector

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O-10-305-1

Modification of horseradish peroxidase and its conjugation to morphine for ELISA development

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The aim of this investigation was developing an ELISA method by monoclonal antibody for detection of morphine in urine in order to identify addicted persons. In this study, peroxidase was used as a label for measurement of morphine. Peroxidase modification was performed by conjugation of an amino group via carbohydrate peroxidase oxidation followed by addition of ethylene diamine. Then morphine was converted to 6-MHS derivative and conjugated to modified peroxidase to produce 6-MHS-Peroxidase. The monoclonal antibody was purified from the culture medium and the titre of antibody and enzyme conjugate were determined by checkerboard titration. The competitive ELISA was performed by using a 1:15 dilution of enzyme conjugate and coated antiboby at 9μg/ml. Sensitivity of this method was 150ng/ml in urine. The antibody cross reacted with codeine and apomorphine phosphate (150 and 11.5 percent respectively) but did not show any cross reactivity with heroine, papaverine, naloxone and naltroxone. Maximum CV of inter and intra assay were 6.2 and 11.14 percent, respectively. The correlation between developed ELISA and immunochromatography was %98.57.

Keywords: ELISA, morphine, peroxidase

P-10-967-1

Steroid receptor RNA activator (SRA) gene expression knockdown by microRNA adapted short hairpin RNA (shRNAmir)

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Steroid receptor RNA activator (SRA) is a coregulator of steroid receptor transcriptional activities and enhances estrogen receptor(ER) transactivation by several folds. Since there is an increase in SRA expression in breast tumor in comparison to normal adjacent tissue, it has been suggested that SRA may play a role in cancer cells. In this study, we designed and developed a RNAi expression vector, microRNA adapted short hairpin RNA (shRNAmir), that mimics microRNA pathway inside the cell. shRNAmir-SRA was specific to SRA and constructed by SOE-PCR and cloning techniques then transfected into human breast cancer (MCF7) cells. SRA expression was estimated by Real Time PCR after two weeks selection with neomycin antibiotic. Our data showed about 60-70% expression reduction of SRA construct compared to control cells. From the obtained result we found that the shRNAmir-SRA could successfully knock down the expression of the target gene and may be suitable for a variety of applications, including tissue-specific knockdown and in vivo forward genetic screens. Further complementary studies are under progress in this regard.

Keywords: RNAi, steroid receptor activator (SRA), breast cancer, shRNAmir

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P-10-988-1

Relationship between life expectancy and the incidence of Motor Neuron Disease

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Adopting a novel approach combining individual-level data on motor neuron disease (MND) cases with aggregate data on age-specific death rates, this paper presents a competing risk analysis of death from MND and death from other causes. The estimated unconditional hazard of death from MND (i.e., if other causes of death were eliminated) is sickle shaped. This is consistent with the theory that susceptibility to MND varies over the population. It is also estimated that the MND mortality rate increases by about 6-7% for one year increase in life expectancy.

Keywords: MND, mortality

P-10-1004-1

Biosensor technologies in drug discovery and medical applications

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The ability to detect pathogenic and physiologically relevant molecules in the body with high sensitivity and specificity offers a powerful opportunity in early diagnosis and treatment of diseases. Biosensors are devices that can detect or quantify molecules of interest and convert a biological response in to an electrical signal. Sensing occurs when there is an interaction between the target molecule and a biological macromolecule (e.g. enzyme, antibody, receptor or DNA strand). In this article classes of biosensors, design and their medical applications have reviewed. One of the most important applications of biosensors is clinical diagnosis and biomedical monitoring. In drug discovery applications biosensors can quickly measure how well a potential drug binds to a target and, by eliminating markers biosensors eliminate potential causes of interference. Biosensors can be classified either by the type of biological signaling mechanism they utilize or by the type of signal transduction they employ. Biosensors have a wide variety and applications in medicine, such as detecting antigens, small molecules and ions in living things: H^+, K^+, Na^+, CO_2, H_2O_2, DNA hybridization and damage, cholesterol, and glucose in diabetes. Currently, cancer can also be detected by monitoring the concentration of certain antigens present in the bloodstream or other bodily fluids.
Keywords: biosensor, drug, medical applications

P-11-1010-1

Applications of Fourier Transform Infrared Microspectroscopy in studies of normal and cancer human gastric tissue

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Fourier transform infrared (FT-IR) microspectroscopy has shown promise as a diagnostic tool for distinction of normal and cancer tissues. IR spectroscopy opens new and modern areas of medical research, as it causes no damage of the cells. In the present work, FT-IR is used to study IR spectral characteristic with respect to cancer and normal tissues from formalin fixed gastric biopsies from four patients. The samples from four patients were removed during surgery and then classified as normal and cancerous. Classification was based on histopathological examinations. Specific regions of the spectra were analyzed to study variations in metabolites. Signified between the two pathological conditions, normal and cancer, changes in relative intensity of the bands in the range of 600-4000 cm-1 were analyzed. Maps of absorbance intensity ratios of bands in the region from 2800-3100 cm-1 and 900-1800 cm-1 have been created in order to analyze pathological changes in tissues. The limited data available showed normal gastric tissue has a strong absorption than cancer types over a wide region in four patients. Detailed analysis showed that there is a significant decrease in total biomolecular components for cancer tissue types in comparison to the normal.
Keywords: FTIR microspectroscopy, gastric cancer, normal and cancerous tissue.

P-10-1022-1

Optimizing of measurement of Aflatoxin-Albumin adducts in serum of rats treated with Aflatoxin B1

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The aim of this study is the optimizing of HPLC method for measurement of Aflatoxin-Albumin adducts as an important marker of Aflatoxin exposure. In this project, three groups of rats were used as positive, negative and standard controls. 0.5mg/kg of Aflatoxin B1 was injected to positive control rats, and 10microCi radioactive Aflatoxin B1 was injected to every standard rat. After getting blood samples from all rats and separating their serum, Albumin was isolated using Ammonium Sulphate and acetic acid, and the purity of Albumin was measured using SDS-Page Electrophoresis, and the concentration of Albumin for all three sample groups was measured using Bradford method. After hydrolyzing Albumin by Pronaz enzyme and passing from Affinity Chromatography column, for purification of Aflatoxin, the sample was injected into HPLC (C18 Column), and the amount of Aflatoxin-Albumin adducts were measured in comparison to standard samples and with fluorescence detector. According to the experiments performed, the purity of the isolated Albumin from serum of rats was found as 97% and the concentration of the purified albumin from serum of positive and negative control and standard rats was 1, 1.3, 1.2 mg/ml, respectively. In HPLC method, detection limit for measurement of aflatoxin was determined as 20pg/mg Alb, and the specificity of the method was determined as more than 90%. The average amount of Aflatoxin bound to Albumin in serum of rats treated with Aflatoxin 12ng/mg Albumin was calculated, and the reproducibility of the method investigated after several times was good. In this method, by the changes made in the percentage of solvents and mobile phase and run time, the measurement was done more easily and quickly. Therefore, it is suggested that HPLC can be used as a sensitive, precise and specific method, and with the improvement made it can be used more quickly, easily and with higher reproducibility for measurement of this important marker for exposure to Aflatoxin in high risk individuals specially in provinces in the country where they use dried nuts in larger amounts.

Keywords: Aflatoxin-Albumin adduct, HPLC

P-10-1099-3

Human Papillomavirus DNA detection in sperm by PCR methods for diagnostic markers

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Our aim was to detect human papillomavirus (HPV) in semen and find diagnostic markers for complex diseases if sperm washing removes HPV DNA. Amplification by nested polymerase chain reaction (PCR) was used to detect viral DNA sequences in semen samples from complex diseases volunteers. Forty-five men had historical or clinical evidence of genital HPV infection (study group) and 39 were healthy, clinically HPV-negative semen donors. We detected HPV DNA in the sperm cells of 24 of 45 subjects (53%) with past or current HPV infections in contrast to three of 39 healthy subjects (8%), ($P<0.001$). Overall, PCR detected HPV in 21 of 32 subjects (66%) with identifiable lesions and six of 53 (11%) without them ($P<0.001$). Swim-up washings of all 27 prewash sperm cells with HPV reduced cellular HPV DNA below detectable levels in only two cases. HPV is present in sperm cells from infected diagnostic markers and apparently healthy subjects, and sperm washing does not eliminate the risk of HPV transmission to recipients. We suggest that HPV DNA testing should be done on the semen of prospective donors, and those with positive tests should be excluded from donation.

Keywords: sperm, cancer, human papillomavirus, neoplasia

P-10-1113-1

Gelatin extraction from the non-treated (Lim- Skin) and treated (Wet Blue Skin) cattle skin

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Gelatin is a protein produced by partial hydrolysis of collagen extracted from the bones, connective tissues, organs, and some intestines of animals such as the domesticated cattle. It has been commonly used as a gelling agent in food, pharmaceutical, and photography. In the present study, gelatin was extracted from the non-treated (Lime- Skin) and treated (Wet Blue Skin) cattle skin. At first, the skins were cut into the small pieces and incubated in the Ca(OH)2 solution (20g/ml) for twenty days. Then, the skins were washed with sulfuric acid and deionized water. The final extraction was done with water at different temperatures (55 –95°C) and the resulting extractions were

concentrated. The products were analyzed on the SDS-PAGE, the tanic acid and sulfate copper experiments were also done on the samples by the Aria gelatin factory. The results have been shown that the percentage of the gelatin extracted from the non-treated skin is more than the treated skin. The percentage of the gelatin extracted was decreased by increasing temperature of the extraction in the non-treated skins. On the contrary, the percentage of the gelatin extracted was increased by the elevating the extraction temperature in the treated skins and the maximum percentage gelatin extraction was at 95˚C. The SDS-PAGE analysis has been shown that the pattern of the mobility of the extracted gelatins is the same as the gelatin extracted from the bone by the Aria gelatin factory. The maximum percentage gelatin extraction was at 55˚C from the non-treated skins.

Keywords: gelatin extraction, lime-cattle skin, wet blue cattle skin