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HEALTH SCIENCES

VALIDATION OF TWO STEP DIFFERENT EXTRACTION FROM SEMINAL FLUID MIXED WITH EPITHELIAL CELLS

GAYVELINE C. CALACAL[1], TANYA ALETHEIA P. BAGADION[2], RALHP KEVIN BRETT[2], JOHN MICHAEL L. DANAO[2], NINA MARIE C. FLETA[2], JOSE CARLO Z. NATIVIDAD[2] and SATURNINA C. HALOS[1]

[1]*DNA Analysis Laboratory, Natural Sciences Research Institute University of the Philippines Diliman, Quezon City*

[2]*Philippine Science Health High School Agham Road, Diliman, Quezon City*

DNA typing of biological material has become one of the most powerful tools for personal identification and has gained worldwide acceptance since 1985. With recent advances in DNA typing, the UP-PACC DNA Analysis Laboratory was established to develop the national capability to respond to this growing need for accurate identification. One of the most common sources of DNA samples obtained from sexual assault cases is a mixture of seminal fluid and epithelial cells. To initiate advancement in the investigation of such cases, this study was performed to assess the feasibility of current protocols in extracting DNA from such biological sources. These protocols on differential lysis were tested. A single mixture of fresh semen and epithelial cells was utilized for conformity in all the three protocols. After the DNA was extracted from the female and male fractions, it was amplified at STR locus HUMFOLP3 (HUMDHFRP2). Analysis of the amplified DNA showed complete separation of the two fractions. All three protocols were suitable for DNA extraction, however, use of the modified FBI protocols yielded higher amount of the needed substance (100μg/mL). Alleles were detected and the sources of the mixed stain were cnfirmed by matching the results with corresponding female and male blood donors.

Keywords: seminal fluid w/ epithelial cells, human identification, two-step differential extraction procedures DNA typing, male/female fractions, differential lysis. PCR, HUMDHFRP2, HUMFOLP23, STR

BIOASSAY OF PARALYTIC SHELLFISH POISONING (PSP) TOXINS FROM TOXIC BACTERIA *(Micrococcus sp.)* USING BRINE SHRIMP *Artemia salina* (Linnaeus)

DEU JOHN M. CRUZ[1,2] and NOEL G. SABINO[2]
[1]*Research and Biotechnology Division*
St. Lukes Medical Center, Quezon City

[2]*Institute of Biological Sciences*
University of the Philippines Los Baños, College, Laguna

Paralytic Shellfish Poisoning (PSP) toxins is a group of related neurotoxins derived from toxic single-cell planktonic organisms known as dinoflagellates. Mollusks infected with any of these organisms may cause food poisoning when ingested. Different bioassay techniques have been developed to determine toxicity levels of mollusks infected wth these dinoflagellates. Studies have also shown a relationship between toxic dinoflagellates and bacteria associated with them. In this study, toxic bacteria (*Micrococcus sp*). isolated from the toxic dinoflagellate *Pyrodinium bahamense var. compressum* was cultivated for production of PSP toxins. The toxins were extracted and identified using spot test and thin layer chromatographic (TLC)- Fluorometric method. Two different bioassay techniques were used to detemine toxicity levels of the extracted toxins. Using the mouse bioassay technique, BALB/c mice were injected intraperitoneally with 1 mL of crude extract while in *Artemia salina* bioassay, 0.5 mL of the crude extract or its dilutions were added to four plates of saline medium containing 10 brine shrimps *(A. salina* nauplii) each. Toxicity levels for mouse and *A. salina* were measured in terms of mouse units and mortality rate, respectively. Spot test of the extract showed a retardation factor (Rf) of 0.79. Death did not ensue in mice and consequently, toxicity in terms of mouse units could be determined. In *A. salina*, addition of the crude toxin extracted to the medium resulted to mean mortalities of 87.14%. Further dilution of the toxin extract to 100 folds resulted to mean mortalities of 43.91%. Further studies are being done to determine concentration and biological activity of the crude extract, as well as to develop a Lethal Dose 50 (LD_{50}) for the *A. salina* bioassay.

Keywords: paralytic shellfish poisoning toxins, spot test, thin layer chromatography-flourometry, retardation factor, intraperitoneal injection, bioassay, mouse unit, mean mortality, lethal dose 50, *Artemia salina nauplii*

DETECTION OF *Mycobacterium tuberculosis* BY POLYMERASE CHAIN REACTION

JINGLE R. CANDELARIO, MA. LOURDES S. YANGA, REENA KATIGBAK-POMPA, LORETO CODAMOS, EVELINO N. LAGAMAYO, ROBERTO BARZAGA, BLANQUITA B. DE GUZMAN, and TB STUDY GROUP
[1]*Research and Biotechnology Division*
St. Lukes Medical Center, Quezon City, Philippines

A polymerase chain reaction assay was used to amplifyh a 123-bp region of the IS 6110 insertion element, in which multiple copies occur n the mycobacterial genome, for the diagnosis of pulmonary tuberculosis. Sputum samples from patients with clinical diagnosis of pulmonary tuberculosis, were evaluated by PCR. Results were compared with those obtained by microscopic examination of concentrated smear stained with acid-fast-kinyoun stain, and radiometer (BACTEC) culture. All smear and culture positive samples were PCR positive, indicating that PCR could be a good tool for the rapid diagnosis of mycobacterial infectious diseases.

Keywords: polymerase chain reaction, *Mycobacterium tuberculosis*, IS 6110, insertion element, acid-fast, kinyoun stain, radiometric, BACTEC culture, mycobacterial genome, infectious diseases

MICROSATELLITE DNA POLYMORPHISMS IN FILIPINOS

JASMIN JIJI B. MIRANDA[1], JOSEPHINE Y. CHU[2], ALLAN CHRIS M. FERREON[1], MICHELLE MUSIC F. MAGNO[2] and SATURNINA C. H ALOS[2]
[1]*Molecular Biology and Biotechnology Program, College of Science*
[2]*DNA Analysis Laboratory, Natural Sciences Research Institute*
University of the Philippines Diliman, Quezon City

Microsatellite DNA are soft tandemly repeating polymorphic sequences distributed throughout the human genome. Genetic variation of microsatellite loci have been studied extensively in many populations worldwide for phylogenetic analysis, genome mapping and linkage analysis, and identity testing in the medical and forensic scienes. This study reports genetic variation in three Philippine enthnolinguistic populations based reports on allele frequencies at the HUMF13AO1, HUMFES/FPS and HUMvWA STR loci.

Genomic DNA samples of consenting individuals from the Cebuano, Ilocano, and Pampango language groups were analyzed using polymerase chain reaction and automated fluorescence-based product detection. While all three Philippine populations were found to share the most frequent alleles at these loci, interpopulational variation was observed on the frequency distribution of the other alleles. Comparison with other Asia Pacific allele frequency data showed significant differences, indicating that these microsatellite loci applicability of the allele frequency data for forensic identity testing, calculations for ramdon-match probability, likelihood ratio, exclusion power of the loci, and power of paternity exclusion were performed. Values suggest that the data generated can be contributed to the national genetic database to be used as basis for forensic casework in the country.

Keywords: human genetic variation, microsatellite DNA, Philippine populations

IDENTIFICATION OF HCV GENOTYPES IN FILIPINO POPULATION BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM

BENJAMIN GERARDO A. PEREZ[1], SHERRIE QUERUBIN DE OCAMPO[1], JOSEPH C. BOCOBO[1], LEONORA TRINIDAD D. SALDA[2], JESUS Y. PEREZ[1], and RONALD R. MATIAS[2,3]

[1]*Institute of Digestive Diseases and* [2]*Research and Biotechnology*
St. Luke's Medical Center, Quezon City

[3]*Institute of Biology, College of Science*
University of the Philippines Diliman, Quezon City

Hepatitis C virus (HCV) is recognized as the major etiologic agent of most cases of acute and chronic non-A, non-B liver diseases and infects around 1% of the general population worldwide. At least nine major genotypes have been documented and a significant number of data indicate that correlation exists between HCV genotypes with clinical course, virulence, and response to interferon therapy. This study aims to identify existing genotypes in the Filipino population by restriction endonuclease cleavage of the RT-PCR amplified 5' non-coding region of the HCV genome. Renal transplant patients, blood donors, and patients undergoing hemodialysis and blood transfusion were included in the study if their serum was found reactive with anti-HCV by second generation EIA. RNA from serum was extracted and a reverse transcription RT-PCR was performed in a single tube assay using nested primers from the highly conserved 5' non-coding region of the HCV genome. Restriction digestion using (a) *Rsa*I and *Hae*III, (b) *Hin*fI and *Mva*I, and (c) *Scr*FI was carried out on secondary PCR products. After electrophoresis through a 3% agarose gel, restriction patterns were determined by comparing digestion products with established genotypes.

Preliminary results show that out of 29 serum samples reactive with anti-HCV by ELISA, 14 were confirmed positive by RT-PCR. Subsequent RFLP analysis of 14 samples identified 12 as genotype 1, 1 genotype 2 and 1 genotype 4. Our ongoing work is designed to correlate the identified HCV genotypes with the degree of liver derangement, biochemically and histologically.

Keywords: Hepatitis C. Virus, HCV genotypes, interferon therapy, restriction endonuclease cleavage, RT-PCR, restriction digestion, restriction fragment length polymorphism, second generation EIA, single tube assay, nested primers

BINDING PROFILE TO NEOGLYCOPROTEIN PROBES IN CERVICAL CARCINOMAS

ANNABELLE A. HERRERA, SONIA JACINTO, DELISA MANUEL, LAURIE JOY VALERA, EDNA AMPARADO
Institute of Biology, College of Science
University of the Philippines Diliman, Quezon City

When affected by oncogenic cells, layers of the cervix exhibit changes in structure and composition. Histochemical changes manifesting qualitative and quantitative alterations of cellular and extracellular glycoproteins may be detected using biotinylated probes. Histochemistry through probe-receptor-color reaction using lactose, mannose, N-acetylgalactosamine, N-acetylglucosamine, as well as hyaluronic acid and fucoidan allowed detection of receptor sites in the tissues obtained from Metro Manila hospitals. Cancer tissues showed greater affinity to lactose while normal tissues showed positive reaction to mannose. Binding profile to the outer probes was highly variable. Lactose and mannose are feasible biomarkers for carcinoma and normal cervical tissues, respectively.

Keywords: binding profile, cervical carcinoma, lactose, mannose, fucoidan, hyaluronic acid, N-acetylglucosamine, N-acetylgalactosamine

BINDING PROFILE OF A LECTIN AND NEOGLYCOPROTEIN PROBES ON NORMAL AND THYROID CARCINOMAS FROM FILIPINO PATIENTS

SONIA D. JACINTO, KATHRYN T. SANCHEZ,
MARIE ANTONETTE H. SUPLIDO, MARIA LORETA V. QUITORIANO,
MARNIE GRACE I. SONICO, ANNABELLE A. HERRERA,
EDNA A. AMPARADO, and ARLENE A. CASAUAY
Institute of Biology, College of Science
University of the Philippines Diliman, Quezon City

Although mortality due to thyroid cancer is not really high, very little is known about it except for its prevalence among Filipino women. Moreover, epidemiological studies of thyroid cancer in the Philippines reveal that incidence of the disease has been on the rise in the past decade. In its respect, thyroid tissues representing 20 normal and 34 papilliary carcinomas were processed for histochemical staining using biotinylated concanavalin A, a mannose-specific lectin and six neoglycoproteins also biotinylated and conjugated to BSA-made histochemically inert. Localization of receptors to the probes was made possible by using peroxidase conjugated avidin-biotin complex and the chromogenic substrate DAB. Results revealed that the probes, concanavalin A is a very promising histochemical marker for transformed thyroid. All of the normal specimens did not stain with the probe but nearly 60% of the thyroid papillary carcinoma were positive for con A receptors. Thus, mannose containing receptors, which are undetectable in normal thyroid gland epithelia, are expressed by cancerous epithelium. The signal becomes stronger in more advanced carcinomas. This information can be used for diagnostic purposes as well as in the development of therapeutic tools for thyroid cancer.

Keywords: lectin, concanavalin A, avidin-biotin complex, papillary carcinoma

BREAST CANCER WITH P53 GENE MUTATION

EDNA A. AMPARADO*, ARSENIA A. CASAUAY*, CUSTER DEOCARIS**,
ANNABELLE A. HERRERA* and SONIA D. JACINTO
**Institute of Biology, College of Science*
University of the Philippines, Diliman, Quezon City

***Philippine Nuclear Research Institute*
Diliman, Quezon City

This study is the first report on the histopathology of breast tissues in Filipinos who have mutation in p53 gene. Patients from the Philippine General Hospital

(PGH), East Avenue Medical center, Veterans Memorial Medical Center were screened by the team from the Philippine Nuclear Research Institute for p53 mutations. Both qualitative and quantitative histopathologic parameters were identified and recorded.

Analyses show that 86% of the Filipino patients have invasive ductal carcinoma, 6% have invasive globular carcinoma, 6% have phallodes tumor, and 2% have mucinous carcinoma. Ductal carcinoma exhibits nuclear features which are markedly different from those of Caucasians. These may be the ethnic-specific profile of Filipino patients. These histopathologic qualitative and quantitative parameters are our original contribution to supplement the morphological routine in malignancy grading.

Keywords: breast cancer, p53 gene, histopathology, carcinoma, malignancy, grading