HEALTH SCIENCES

HSD No. 1 DEVELOPMENT OF AN IMMUNOFLUORESCENCE ANTIBODY TEST FOR THE DETECTION OF ANTI-*Cryptosporidium parvum* ANTIBODIES IN HUMAN SERUM SAMPLES

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An Immunofluorescence Antibody Test (IFAT) for the detection of anti-*Cryptosporidium* antibodies in serum samples was developed to make diagnosis of cryptosporidiosis less expensive, faster, and easier.

The dilutions of the blocking solution, serum sample, mouse anti-human MAb, and washing solution were tested for optimal fluorescence under both positive and negative sera. Antigen obtained through immunomagnetic separation and the use of anti-human IgG was also tested. Fifty-nine samples from the Pediatric Ward and Cancer Institute of the Philippine General Hospital were tested for seropositivity using the developed technique.

The optimal dilutions determined were goat serum diluted 1:3 in PBS for blocking solution, serum diluted 1:5 in blocking solution, mouse anti-human MAb diluted 1:50 in blocking solution, and 0.05% Tween-20 in PBS for washing solution. Using purified oocysts as antigen was also found to significantly decrease errors in observation of fluorescence. Multivalent mouse anti-human MAb were more effective in detecting positive sera than anti-human IgG. Among the fifty-nine samples from the Philippine General Hospital 27% were seropositive using this technique. Intense fluorescence results most probably from sera in patients about 32-60 days past infection. Those with low titers associated with an infection past more than 60 days constituted 36% of the population. Among the females, 30% were seropositive and among the males, 24% were seropositive. This procedure revealed high antibody titers in asymptomatic cases, which indicates a high occurrence of *C. parvum* infection and could be carriers.

This IFAT may be used to assess and treat members of communities suspected to be a site of a cryptosporidiosis outbreak to prevent reinfection through untreated carriers. Also, it may be used as an epidemiological tool to evaluate the hygienic practices and water cleanliness of an area.

Keywords: *Cryptosporidium parvum*, Immunofluorescence Antibody Test (IFAT), diagnosis, epidemiology, seropositivity, oocysts, monoclonal antibodies.

HSD No. 2 IMMUNOFLOURESCENCE ASSAY IN THE DIAGNOSIS OF DENGUE VIRUS INFECTIONS: A PRELIMINARY STUDY

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Dengue fever and dengue hemorrhagic fever (DF/DHF) are among the most common infectious diseases transmitted by the mosquito vector *Aedes aegypti*, in both tropical and subtropical regions. This study provides initial data on the application of buffy coat and the monoclonal antibodies generated against dengue virus prepared at RBD-SLMC, the confirmation of dengue infection by Immunoflourescence assay (IFA).

Buffy coat was taken from the centrifuged whole blood of the suspected dengue patients from San Lazaro Hospital. Cells from the buffy coat were stained with monoclonal antibodies against dengue and visualized by FITC-conjugated anti-mouse antibody. Furthermore, plasma and/or RNA extracted from the plasma were used as template in an RT-PCR method for the amplification and detection of dengue viral genome. Plasma was also inoculated to C6/36 *Aedes albopictus* cell lines and after two weeks post-infection the culture fluid was again used for sandwich ELISA to detect dengue virus.

Twenty-five percent (25%) of thirty-eight (38) samples out of 152 were positive for dengue virus using monoclonal antibodies and buffy coat. The results were correlated with other methods such as RT-PCR, antigen sandwich ELISA, and virus isolation method using C6/36 *Aedes albopictus*.

Immunoflourescence using monoclonal antibodies and buffy coat is shown to be a faster way of detecting dengue virus compared with conventional methods.

Keywords: Buffy coat, dengue virus, Immunoflourescence assay, RT-PCR, sandwich ELISA

HSD No. 3 IDENTIFICATION OF NEURALIZING MONOCLONAL ANTIBODIES AGAINST DENGUE VIRUSES

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Identification of dengue virus neutralizing monoclonal antibodies is essential for

developing potential anti-dengue therapeutic agents. In this study, a panel of monoclones was generated and antibodies produced were characterized for their ability to neutralize the dengue virus.

Ascitic fluids containing high titers of monoclonal antibodies were obtained from BALB/c mice previously injected with hybridoma cells. The ascitic fluids were titrated and screened for their neutralizing activity agains the four dengue virus serotypes using Plaque Reduction Neutralization Test (PRNT) and Tetrazolium Dye Reduction (MTT) Assay. Serial dilution of the monoclones were mixed for one hour at 37° C with a known dengue virus titer. The mixtures were inoculated into LLcMK$_2$, either in 35 mm petri dishes or 96-well microtiter plates.

Using the MTT assay, three monoclonal antibodiesm 12D11/7E11, 12D11/9F5 and 12D11/10C3 that were earlier shown to be reactive to the four dengue serotypes by enzyme linked immunosorbent assay inhibited the growth of two isolates of dengue virus serotype 1,22St-12A and 9D-36, up to dilutions of 5000.

The monoclonal antibodies used in this study were found to inhibit dengue virus multiplication hence have neutralizing properties. These may be used to identify and characterize epitopes on the surface of dengue viruses that are essential for virus infection. Such information is vital in the development of effective treatments or vaccines against dengue virus.

Keywords: monoclonal antibodies, dengue virus, neutralizing antibody, plaque reduction neutralization test, MTT assay, anti-viral

HSD No. 4 CHROMATOGRAPHIC ANALYSIS OF SHABU AND ECSTASY IN URINE SAMPLES

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Currently, there is an increasing drug problem brought about by the illegal use of shabu and ecstasy. This study meets the need to develop a more sensitive and accurate method of detecting these dangerous drugs in biological fluids.

Three chromatographic methods, Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GCMS), were developed to detect shabu and ecstasy in urine samples.

For TLC, the extract of both drugs was chromatographed in ethyl acetate/methanol

water using the Toxilab system. In the HPLC analysis, an Alusphere column was used with acetonitrile and phosphate buffer at pH10 as mobile phase with detection at 215 nm. And prior to GC/MS analysis, the drug extract was derivatized using MSTFA under nitrogen gas. Electron impact was used as the ionization mode.

For TLC method using the Toxilab system, both drugs have the same R_f at 0.23 but by using different developing solutions shabu and ecstasy could be differentiated. The HLPC method developed could separate and quantitate the two drugs. Shabu had an average retention time of 9.9 min while ecstasy was at 7.5 min. The GCMS analysis gave the retention time of 6.31 min for shabu and 10.14 min for ecstasy. As a confirmation technique, the diagnostic ions used for shabu were m/z 91, 130, 206 while m/z 130, 250 was utilized for ecstasy. Linear response for both drugs was in the concentration range of 250 to 1000 ng/ml.

The chromatographic methods developed could be used for the routine hospital screening and confirmation of shabu and ecstasy in urine.

Keywords: shabu, ecstasy, chromatographic analysis. urine samples, dangerous drugs

HSD No. 5 LABORATORY DIAGNOSIS OF CENTRAL NERVOUS SYSTEM VIRAL INFECTIONS BY POLYMERASE CHAIN REACTION

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Research, epidemiological studies and laboratory analysis of viral diseases of the central nervous system have been few in the Philippines. Thus, most of the clinically suspected viral encephalitis or meningitis cases are treated without etiological laboratory confirmation. This study was done to show the presence of some human herpesviruses, such as herpes simplex viruses 1 and 2, varicella zoster virus, cytomegalovirus, and Eipstein Barr viruses 1 and 2, in cerebrospinal fluid samples taken from Filipino patients diagnosed with viral encephalitis or similarly related conditions.

The Polymerase Chain Reaction technique has provided a highly sensitive and

specific method of directly detecting the viral genome in an infected sample, such as cerebrospinal fluid. In this study, viral DNA was extracted from CSF by enzymatic digestion with proteinase K, followed by phenol-chloroform extraction and washing with ethanol. PCR was performed for each of the following viruses at varying conditions: HSV-1 and 2 with a target sequence from the glycoprotein D gene of 271 bp, VZV with a target sequence from Gene 29 of 200 bp. CMV with a target sequence from the major immediate early gene of 435 bp, and EBV with a target sequence from the EBNA-3C gene of 153 bp for type 1, and 246 by for type 2.

Out of 234 samples analyzed, 9 were HSV-1, 7 were HSV-2, and one contained both HSV-1 and 2. Out of 213 samples examined, 8 were co-infections in varying combination of HSV 1 and 2, EBV1 and 2 and VZV. Out of 213 samples, two were VZV and out of 128 samples, 60 were EBV 1 and all EBV 2 were co-infected with another virus. All in all 31 samples were infected with more than one virus.

The laboratory diagnosis of CNS viral diseases has been facilitated by PCR. IT has been shown to be a rapid, sensitive, and specific enough to be used as a frontline test for the detection of viral DNA in CSF, eliminating the need for difficult to obtain, expensive, and highly invasive brain biopsy which is the gold standard.

Keywords: Central nervous system, cerebrospinal fluid, Herpes simplex virus, Varicella zoster virus, Epstein-Barr virus, Polymerase Chain Reaction

HSD No. 6 PRE-CLINICAL EVALUATION OF SAFETY OF A POTENTIAL DNA VACCINE AGAINST MALARIA

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DNA vaccines represent a novel method of vaccination, and are extremely promising for application and development in developing, tropical countries like the Philippines. However, like all technologies, there are risks associated with the use of DNA vaccines. These risks include possible integration of the DNA vaccine into the host genome, and immunopathology and histopathology due to the vaccine. We have constructed a putative DNA vaccine for malaria (VR12MM1), and we have performed a pre-clinical evaluation of this vaccine in mice.

DNA vaccines were prepared by overlap extension Polymerase Chain Reaction (PCR) to create the multi-epitope insert, and by recombinant DNA technology to create the recombinant plasmid, the DNA vaccine. Safety was determined by employing a PCR assay to assess persistence of the DNA vaccine in tissues of immunized mice, and to

determine possible integration of the vaccine plasmid into the mouse genome by homologous recombination. Histo-pathological analysis was also conducted on tissues from immunized animals.

Plasmids were not found to persist in immunized animals for more than a week, and no integrated vaccine sequences were found in the mouse genome. No significant adverse effects of vaccination were found by histopathological analysis.

Keywords: DNA vaccine, safety, malaria, integration, homologous recombination, PCR, histopathology

HSD No. 7 EFFICACY OF *Mycobacterium vaccae* AS AN UMMUNOTHERAPEUTIC AGENT IN THE MANAGEMENT OF PULMONARY TUBERCULOSIS

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Patients who consulted at the TB Clinic of the Section of Infectious Diseases, Department of Medicine, UP-PGH and the Quezon Institute from January 1993 to June 1997 with microbiologically proven pulmonary tuberculosis based on a positive AFB smear and culture were included in the study. A total of 78 patients were classified as a multi-drug resistant TB or complicated TB. Both of the susceptible and multi-drug resistant tuberculosis were randomly assigned to either the immunotherapy (given an intradermal injection of Mycobacterium vaccae) of the non-immunotherapy group (without the intradermal injection of *Mycobacterium vaccae*).

Using the time for sputum conversion to negativity in weeks as primary outcome, all of the patients were followed-up. Results show that for the susceptible or uncomplicated tuberculosis, there is an earlier conversion of sputum smear and culture to negativity in patients given the immunotherapy as compared to those who did not receive immunotherapy (51.16% vs. 40.00% at two weeks respectively). Conversion rates, however, became comparable by the 12th week of chemotherapy (90.70% vs. 88.57% respectively).

Secondary parameters of weight gain were likewise higher for the Immunotherapy Group as compared to the Non-Immunotherapy Group. For the multi-drug resistant or complicated cases, the mean time for sputum conversion was also compared between immunotherapy and the non-immunotherapy group. Utilizing the Kruskal-Wallis one way analysis of variance, there was statistical significance. When we analyzed the effect of

resistance to each drug on time for sputum conversion to negativity utilizing the chi-square, only etambutol and pyrazinamide were shown to have significance. This means that immunotherapy had a more beneficial effect on earlier sputum conversion when the resistance was to ethambutol or pyrazinamide.

These results demonstrated the potential usefulness of *Mycobacterium vaccae* as an immunotherapeutic agent in the management of both susceptible *M. tuberculosis* or uncomplicated PTB and complicated or multi-drug resistant cases. The addition of Mycobacterium vaccae at one to two weeks after initiation of chemotherapy may lead to earlier sputum negativity by both smear and culture.

Keywords: susceptible, multi-drug resistant, tuberculosis, immunotherapy, Mycobacterium vaccae, sputum smear and culture

HSD NO. 8 THE *flaA* GENE WHICH CONFERS PARTIAL PROTECTION IN MICE AGAINST *Helicobacter pylori* IS DETECTED BY PCR IN PHILIPPINE STRAINS OF *H. pyroli*

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The flagella of *Helicobacter pylori*, a bacterium which causes chronic gastritis, is considered as one of the principal virulence factors of this human pathogen. The *flaA* gene is the gene which encodes the major glagellin in the flagellar sheath of *H. pylori*. It was recently discovered in the US that generic immunization using fla A gene confers partial protection against H. pylori in mice.

The objective of this study was to determine by PCR the presence of the *flaA* gene in *H. pylori* strains isolated from gastric biopsies of Filipino patients diagnosed to have various gastroduodenal diseases at St. Luke's Medical Center.

H. pylori strains were isolated, cultured and purified from gastric biopsies of 20 patients suffering from different gastroduodenal diseases. Two patients were suffering from dyspepsia, seven with gastric ulcers, seven with duodenal ulcers, two were diagnosed with aphthous ulcers and two patients with both duodenal ulcers and gastric ulcers. Presence of the *flaA* gene was detected by PCR using the *flaA* primers (forward TTCTATCGGCTCTACCAC and reverse CTGACCGCCATTGACCAT) with a PCR product of 500 bp. The cycling conditions were: 95°C for 5 min, and 30 cycles of 95°C for 1 min, 52°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 10 min was performed.

Out of 20 *H. pylori* strains, 10 were positive for the *flaA* gene: 4 from patients with gastric ulcers, one each from aphthous ulcer, both gastric and duodenal ulcer and dyspepsia.

The results demonstrate that the *flaA* gene could be detected by PCR from some Philippines strains of *H. pylori*. The flaA gene could be isolated and tested as a possible DNA vaccine against *H. pylori*.

Keywords: *Helicobacter pylori*, gastroduodenal diseases, chronic gastritis, *flaA* gene, PCR

HSD No. 9 CHARACTERIZATION OF THE BIOLOGICAL ACTIVITIES OF A 50-Kd PROTEIN PURIFIED FROM *Tinospora rumphii* Boerl

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Tinospora rumphii Boerl, locally known as *makabuhay*, is one of the most common plants being used for various ailments. To characterize the biological activities of the plant, its antimicrobial, hamagglutinating, immunodulating, cytotoxic and apoptosis-inducing properties were investigated.

All experiments were carried out using a purified 50-kD protein from the methanol extract of *Tinospora*. Nitric oxide (NO) production was measured by a microplate assay based on Griess reaction. Hemagglutination (HA) assay was done using human blood types A, B, O and rabbit erythrocytes. A cell culture-based cytotoxicity system had been employed using HT-29 (human colon cancer cell line) and vero (monkey kidney epithelial) cells. Cell survival was measured by using the microculture-MTT assay. *In vitro* antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aaeruginosa* was determined by the agar disk diffusion method. Apoptosis was detected by fluorescence microscopy after staining the cells (HT-29, BHK and vero) with acridine orange and ethidium bromide.

In vitro screening of the plant extract indicated a dose-dependent cytotoxicity for both the vero cells and HT-29 cell line. Results showed that the plant extract stimulated NO release from lipopolysaccharide-activated macrophages. In the antimicrobial screening, the extract demonstrated a strong activity against the Gram (+) bacteria (*S. aureus* and *S. epidermidis*). The extract had a 32 HA units using human blood types A, B, O and rabbit

erythrocytes. The apoptosis assay resulted to a dose-dependent apotosis-inducing activity for the HT-29, BHK and vero cell lines.

Tinospora shows a multiplicity of biological activities. Further work on *Tinospora* should focus on the therapeutic potential of these activities.

Keywords: *Tinospora rumphii* Boerl, immunomodulating activity, *in vitro* cytotoxicity, antimicrobial, hemagglutination property, apoptosis

HSD No. 10 BIOLOGICAL PHENOTYPES OF PLAQUE-PURIFIED DENGUE VIRUSES

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Dengue fever/Dengue hemorrhagic fever outbreaks have been associated with the presence of two or more co-circulating virus serotypes. In this study, the biological phenotype of plaque purified dengue viruses is described.

Dengue viruses were detected and isolated by inoculating patient sera to C6/36 *Aedes albopictus* cell lines. Amplified dengue viruses in the culture were purified by plaque assay. The viruses were inoculated into $LLcMK_2$ (monkey kidney cells) in 35 mm petri dishes. Progeny viruses arising from a single infectious virion were recovered from areas of cell lysis also known as plaques. The method was repeated three times. Dengue serotype was confirmed by R-PCR using type-specific primers. Plaque size and morphology as well as virus titer were also noted.

From 49 dengue virus isolates, 16 were successfully plaque purified. Among them, four were DEN-1, eight were DEN-2, three were DEN-3, and one was DEN-4. Plaque morphology was described based on size and shape. It was observed that small plaques (0.3 to 2mm) showed complete cell lysis from the foci to the periphery, while medium (2.1 to 3.2 mm) and large plaques (>3.2 mm) were characterized by complete cell lysis at the foci and incomplete lysis at the periphery. Both small and medium-sized plaques were observed in all dengue serotypes except for one variant of DEN 2 that exhibited a large plaque size (4 mm). Virus titer ranged from 1×10^3 to 2×10^7 PFU/ml.

Dengue viruses purified in this study exhibited differences in plaque morphology and infectivity titers. No correlation was observed between plaque size and virus titer

Virus growth kinetics is currently being correlated with both plaque morphology and size.

Keywords: dengue virus, plaque assay technique, virus purification

HSD NO. 11 PHYTOCHEMICAL SCREENING OF AN ACCLAIMED HERBAL MEDICINE AGAINST TAGULABAY

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In pursuance of its responsibility to provide adequate and accessible health car to the Filipinos, the use of Philippine traditional and herbal medicines as an alternative cure to common illnesses is being promoted by the Department of Health together with local government units(3). Extract from boiled male Karamay (*Cicca acida* Linn.) leaves was claimed by town folks in Nueva Ecija to be an effective cure for *Tagulabay*, an illness characterized by isolated skin rashes, itchiness and reddening of eyes and skin. Phytochemical screening of the crude extract was done for the preliminary screening of active components that may be responsible for the acclaimed medicinal property. Phytochemical tests revealed that male karamay leaves contain saponins and tannins, both of which are known to have anti-inflammatory properties (1,2).

The anti-bacterial and anti-fungal properties and cytotoxic activity was explored further to determine other possible applications of the putative herbal remedy. However, preliminary tests showed that the extract has no anti-fungal nor an anti-microbial property and is toxic to brine shrimp.

Keywords: phytochemical screening, cytotoxic activity

HSD No. 12 DETECTION OF bcr-abl GENE FUSION BY GTG BANDING AND RT-PCR IN FILIPINO PATIENTS WITH CML

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Chronic myelogenous leukemia (CML) is a hematologic malignancy that is generally characterized by a reciprocal translocation between chromosomes 9 (9q34) and 22 (22q11). This structural aberration results in the formation of an abnormally short chromosome 22 (Ph or Philadelphia chromosome) where the fusion of the genes *bcr* and *abl* is mapped. In this study, cytogenic and molecular techniques were compared in the detection of gene fusion in CML.

Twenty-nine Filipinos clinically diagnosed with CML were studied. Peripheral blood samples or bone marrow aspirates from each patient were cultured. Chromosomes were stained following the GTG (G-bands by Trypsin Using Giemsa) method. Cytogenetic analysis was made following the ISCN (International System for Human Cytogenetic Nomenclature) 1995. Molecular studies were carried out by RT-PCR (reverse transcription – polymerase chain reaction) using G, α , F and A primers.

Cytogenetic analyses revealed the presence of the Ph chromosome in 17 patients. The RT-PCR technique detected the *bcr-abl* gene fusion in 28 patients, 15 had b3a2 gene fusion, 7 were of the b2a2 type of gene fusion and 7 had both types of gene fusion. The detection rate of the RT-PCR technique (96%) is very much higher than that of the cytogenetic method (38%).

Thus, RT-PCR proved to be more sensitive in detecting the *bcr-abl* gene fusion in CML. This technique greatly improves the accuracy and efficiency of the diagnosis of CML.

Keywords: chronic myelogenous leukemia, hematologic malignancy, *bcr-abl* gene fusion, Philiadelphia chromosome, GTG-banding, RT-PCR