major parts, namely: the raking fork assembly, driving mechanism and main frame.

Field tests of the reaper-gatherer showed that the cut crop could be gathered into small bunches similar to what is done in manual reaping. The average distance between adjacent bunches was 2.6 m while the straw bunch was measured 33 cm in diameter. With the use of this device, the labor requirement for gathering a hectare of mechanically reaped rice crop was reduced by 25%, i.e. from 8 to 6 persons.

Keywords: Windrow, Crop gathering attachment, Vertical gatherer, Rake-type gatherer, Horizontal collector with scraper, Reaper-gatherer.

HEALTH SCIENCES

HSD No. 1

PROPHYLACTIC AND ANTIMICROBIAL PROPERTIES OF BREAST MILK: FACT OR MYTH?

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Breast milk, once the only known milk for infants has largely been replaced by a great variety of commercial milk formulas. Reports from various health centers finally prompted the World Health Organization (WHO) to urge mothers to go back to breast-feeding. To contribute to the Philippine government's efforts to this end, we did both laboratory experiments and surveys to obtain concrete indicators supporting the health claims concerning breast milk.

Samples of human breast milk were collected and tested for antimicrobial properties against four potentially pathogenic bacterial isolates. A replicated disc diffusion assay was used to evaluate the extent of the antimicrobial activity of the milk samples. Questionnaires with 30 carefully crafted questions were randomly distributed to 37 respondents, mothers with at least one child either breast-fed or bottle-fed for at least the first six months of life. The prophylactic values of breast milk and milk formula were compared using five commonly encountered childhood

diseases as indicators. The index of prophylactic value of breast milk was also computed for each of the five diseases.

All milk samples significantly showed antimicrobial action on all test organisms. Breast milk also showed a higher prophylactic value either specifically for each disease, or generally when taken as a whole, compared to milk formula. Computed indices of prophylactic value revealed that breast milk surpassed milk formula the most in preventing the occurrence of diarrhea.

Based on our results, we conclude that the general health claims concerning breast milk can be scientifically supported. Breast milk has both desirable antimicrobial activities and prophylactic properties against a number of infections commonly affecting children. The lesser incidence of breast cancer in breast-feeding mothers should be an added incentive to all nursing women.

Keywords: breast milk, antimicrobial, prophylactic value, index of prophylactic value, diarrhea, breast cancer

HSD No. 2

BACTERIOLOGICAL EXAMINATION OF LOCAL DRINKS SOLD IN THE STREETS OF LOS BAÑOS, LAGUNA

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The bacteriology of local drinks ("palamig") sold at selected sites in the streets of Los Baños, Laguna was studied, thirteen samples obtained from 5 types of drinks (namely, buko, melon, sago-gulaman, pineapple and nata-gata) were analyzed for coliforms, total viable bacterial count under mesophilic conditions and for the constituent bacterial microflora.

All of the samples were found to contain coliforms at 37°C; 78% had

detectable coliforms even at 45°C. Mesophilic bacterial counts ranged from 4.4 x 10^4 to 2.3 x 10^6 colony forming units (cfu) per ml. Except for the pineapple juice samples which had the lowest counts, almost all the drinks had high bacterial counts: 10^5 or 10^6 cfu/ml.

A total of 6 (15 gram-positive and 48 Gram-negative) pure bacterial isolates were obtained from the different local drinks samples. Of the 15 Gram-positive isolates, 12 were *Staphylococcus* spp., 2 were *Micrococcus* spp. And one was an unidentified catalase-positive, oxidase-negative, non-sporeforming rod. Majority of the Gram-negative bacterial isolates (34) belonged to Family Enterobacteriaceaen in particular, to the following genera: *Serratia*, *Escherichia*, *Enterobacter*, *Salmonella* and *Providencia/Citrobacter*. Fourteen non-enteric bacteria were identified to be *Pseudomonas*, *Alcaligens/Agrobacterium*, *Aeromona* and *Vibrio spp*. Three were very similar to *Acinetobacter*.

The bacteria most commonly found in the street-sold drinks sampled were: *Starphylococcus, Providencia/Citrobacter,Serratia and Enterobacter*.

Keywords: bacterial microflora, street-sold drinks, coloforms, mesophilic counts

HSD No. 3

PATHOGENIC MICROORGANISMS IN COCKROACHES FROM ILIGAN CITY

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Pathogenic organisms were isolated from cockroaches collected from different households in Iligan City. The most prominent of which is *Vibrio cholerae*. Other microorganisms isolated were bacterial families belonging to Enterobacteriacea and Pseudomonacea depending on the type of environment the cockroaches inhabit. Bacteria associated with cockroaches were also isolated from individuals belonging to the genus Leucophaea and Periplaneta. These associates were tested against the test bacterial organisms *Escherichia coli, pseudomonas aeroginosa*, *Bacillus subtilis*, and *Staphilococcus aureus* as well

as the fungi *Sacchromyces cerevisciae* and *Aspergillus niger*. Results showed that one isolate exhibited antibacterial activity against all test organisms. Implications of the study in disease transmission based on survey conducted on different households are also presented.

Keywords: pathogen, cockroach, *Vibrio cholerae*

HSD No. 4

ISOLATION, CHARACTERIZATION AND DETERMINATION OF BIPHENYL-DEGRADING ACTIVITY OF BACTERIA FROM CONTAMINATED SOIL AND WATER

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Bacteria were isolated from wastewater of a solid waste treatment facility and machine oil-contaminated soil on the basis of their ability to grow on mineral medium supplemented with naphthalene or biphenyl as the sole carbon source. Diluted wastewater and soil slurries were lawned onto non-nutrient agar plates supplemented with naphthalene or biphenyl, and individual colonies were picked and transferred into M9 mineral broth with 0.01% naphthalene or biphenyl. Some isolates were observed to turn the colorless M9 medium into bright green or yellow after incubating overnight at 37°C. Genomic DNA was extracted and the isolates were screened by PCR for the presence of bph genes (A1, A2, A3, A4, B and C) using primers designed from the *Pseudomonas pseudoalcaligenes* KF707 *bph* operon. Isolates that grow in the selection media and that have any of the *bphA* genes were further purified and re-screened for bphA genes. These were then identified using the Crystal ID system from BBL™. All isolates identified so far were gram negative bacilli or cocci belonging to the genera Pseudomonas, Klebsiella, Burkholderia, Acinetobacter, Citrobacter, and Serratia.

Different concentrations of biphenyl in absolute ethanol were scanned

using a luminescence spectrometer (Perkin Elmer LS55) to determine the excitation and emission wavelengths specific for biphenyl. Biphenyl can be detected by this system at a concentration range of 0.001 ug/mL to 0.2 ug/mL. To trace the degradation of biphenyl by the bacterial isolates, tubes containing 100 ug/mL biphenyl in M9 mineral medium were inoculated with selected isolates and incubated at 37 °C. Biphenyl was extracted with an equal volume of chloroform. The chloroform was evaporated under a steady stream of nitrogen gas and the resulting residue was dissolved in an equal volume of absolute ethanol and the samples were read in the luminescence spectrometer. Four strains (Pseudomonas D12e, Pseudomonas I5c, Burkholderia I6b, and Acinetobacter Gr21a) were chosen for their ability to degrade biphenyl from 100 ug/mL to almost zero within 24 hr. The biphenyl-degrading bacteria were characterized as to generation time, rate of biphenyl degradation and growth rate in the presence of high amounts (1000 ug/mL) of biphenyl.

Keywords: biphenyl-degradation, environmental biotechnology, bioremediation

HSD No. 5

RAPID SPECTROFLUOROMETRIC DETECTION OF GALACTOSEMIA

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Galactosemia is a metabolic disorder characterized by the absence of the enzyme galactose-1-phosphate uridyl trasferase (GALT) which is responsible for the transformation of galactose-1-phosphate into glucose-1-phosphate. If undiagnosed and untreated, it could lead to retardation and even death in newborns.

The enzymatic method by Beutler and Baluda was adopted and modified for the assay of GALT. Blood samples from infants were collected on a special filter paper. A blood spot from each sample was obtained and placed in a black wellplate. GALT substrate was added and the blood spot was incubated at 37 °C. After addition of ethanol, the samples were analyzed using the LS55 Perkin Elmer

Luminescence Spectrofluorometer with an excitation wavelength of 355 nm and emission wavelength of 460 nm. With the use of a well plate reader both the calibrators and samples could be simultaneously analyzed unlike in the conventional spectrofluorometer using a one-cell holder in which fluorescent intensity is read one at a time. Linear response is obtained for the concentration range of 2.4 to 16.5 units GALT/ g Hb.

The method was tested using normal and abnormal controls. Thirty blood samples from newborns, 10 blood samples from children and 17 blood samples from adults were analyzed. All samples were found to be negative for galactosemia.

This sensitive and rapid assay requires a very small amount of sample and lesser amount of reagents compared to the classical spectrofluorometric method. It is more reliable than the diagnostic kits. This technique is thus very suited for routine detection of GALT.

Keywords: Galactosemia, galactose 1-phosphate uridyl trasferase, spectrofluorometer, well plate reader

HSD No. 6

DETECTION OF TELOMERASE ACTIVITY IN ADULT LEUKEMIA USING TELOMERIC REPEAT AMPLIFICATION PROTOCOL (TRAP) ASSAY

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Telomeres are the protein DNA structures at the end of eukaryotic chromosomes. It is essential in eukaryotic chromosomes because it protects the chromosomes from degradation and end-to-end recombination. Telomeres are usually replicated by telomerase, a ribonucleoprotein enzyme complex that stabilizes telomere length by adding hexameric TTAGGG repeats. Telomerase is repressed in most somatic cells but is active in germ cells, proliferative renewed tissues and in most cancer cells. Several studies have reported that an up-

regulation of telomerase activity suggests progression from chronic to blast phase in most types of leukemia. In this study, we used a PCR-based assay, the Telomeric Repeat Amplification Protocol (TRAP) to detect telomerase activity. TRAP assay, unlike most PCR-based applications, which measure a fixed amount of nucleic acid target in a sample, a technique that measures an enzymatic activity where the amount of target is dependent upon the biochemical activity of the enzyme. In this study, TRAP assay was performed on bone marrow aspirates from 28 adult Filipino patients clinically diagnosed with leukemia. The assay showed that 20 out of the 28 samples have an up-regulation of the regulation of the telomerase activity.

Keywords: telomere, telomerase, leukemia

HSD No. 7

A COMPARATIVE STUDY BETWEEN THE ST. LUKES\'S IgM-CAPTURE ELISA AND A COMMERCIALLY AVAILABLE IgM ELISA KIT FOR CONFIRMATION OF DENGUE INFECTIONS

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We developed an in-house IgM-capture Elisa for diagnosis of dengue infection at the Research and Biotechnology Division, St. Luke's Medical Center. The procedure makes use of locally isolated dengue viruses as assay antigen and horseradish peroxidase (HRPO) conjugated IgG to Flaviviruses from human high titer pooled serum. We evaluated the performance of this assay by comparing with the imported dengue IgM-capture ELISA kit (PanBio, Australia) which is commercially available in the Philippines. Forty-one (41) IgM positive human serum samples and 44 IgM negative samples were selected from the St. Luke's Dengue Serum Bank.

The sensitivity and specificity of our in-house dengue IgM-capture ELISA were 100% (27/27) and 80% (44-55), respectively. The positive predictive value and the negative predictive value were 65.9% (27/41) and 100% (44/44), respectively. Statistical analysis showed that the agreement rate between the St. Luke's dengue IgM-capture ELISA and PanBio kit was good: k)kappa) value was 0.725 (significant at alpha<0.001). The correlation between the two assays was also good, with correlation coefficient of 0.900 (significant at the 0.01 level, 2-tailed). The St. Luke's Assay can test 45 samples in duplicate per run (including duplicates of positive control, negative control and blank) and costs P1,250.00, while the PanBio kit costs P24,090.00 for similar format. The locally developed kit is more cost effective although it takes two hours longer. However, the additional two hours is insignificant because the results are also obtained within the same day.

Keywords: dengue infection, IgM-capture ELISA, PanBio kit, dengue confirmation

HSD No. 8

CHARACTERIZATION OF *Candida* spp. ISOLATED FROM PAP SMEAR-TESTED WOMEN IN LOS BAÑOS, LAGUNA

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Vaginal swab samples were taken from women who consulted for pap smear-testing in participating clinics in Los Baños, Laguna. The objectives were to isolate *Candida*-like fungi from the pap smear samples, characterize the isolates culturally, morphologically and biochemically and estimate the prevalence of *Candida* species among the subjects.

Twenty-six yeast-like fungi were isolated and purified from 95 vaginal swab specimens but only 20 characteristically produced blastospores and pseudohyphae on Corn meal Agar. Each of these 20 suspected *Candida* isolates was then further characterized for identification based on: colony characteristics on Saboraud Dextrose Agar (SDA) and Broth (SDB), wet mount preparationsof a

3day old-culture grown on SDA, observations of a 2-day old-plate culture and a 4-5 day old-agar slide culture growing on Corn Meal Agar, and fermentation of the sugars dextrose, maltose, sucrose and lactose.

Five of the *Candida* isolates were identified to be *C. albicans*, exhibiting creamy, soft, smooth growth on SDA, forming chlamydospores, pseudohyphae and blastospores on Corn Meal Agar, producing no surface growth on SDB and fermenting dextrose, maltose and sucrose but not lactose. Five other isolates were very similar to *Candida* tropicalis, three resembled *C. stellatoidea* while seven remained unspeciated due to the need for additional tests.

The estimated prevalence of *Candida* species in the study population was 21.2% and its was found to be relatively higher among past users of oral contraceptives, or OC (35.5%) compared to those who never used OC (14.8%). It was also higher among pregnant (42.9%) than among non-pregnant study subjects (19.3%).

Keywords: Candida species, vaginal swabs, pap smear-testing, chlamydospores, pseudophyphae

HSD No. 9

GENOTYPIC ANALYSIS OF CITRIC ACID-PRODUCING FUNGI USING SINGLE STRAND CONFORMATION POLYMORPHISMS (SSCP)

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The study made use of single strand conformation polymorphisms (SSCP) and the ITS1-5.8S-ITS11 (ITS: internal transcribed spacer) region of rDNA in differentiating fungal strains. Citric acid-producing fungi isolated from rotten fruits (*Mangifera indica* and *Citrus reticulata*) were used. Fungal isolation was

done by serial dilution and plating of macerated rotten fruits. Citric acid production of purified isolates was determined by titrimetric and colorimetric assays. Genomic DNA was extracted from fresh mycelial pellets using the CTAB method. PCR was performed using the ITSI-5.8S-ITSII region of rDNA as the target. Four primers, namely, ITS1, ITS2, ITS86, and ITS4, were used to obtain 3 differently sized fragments. PCR products were mixed with formamide loading dye, denatured for 10 min at 95°C, snap chilled on wet ice, and then ran on 6% polyacrylamide gel at a constant current of 5mA.

Six isolates were obtained and were putatively identified as *Aspergillus* strains based on morphology and cultural characteristics. The sizes of the amplicons are 600, 270, and 300 bp for ITSI-5.8S-ITSII, ITSI, and ITSII fragments, respectively. SSCP results show that the isolates have very similar, if not identical, banding patterns with *Aspergillus niger* compared with other control strains under the same genus. This indicates that the isolates belong to the same species of *Aspergillus* and this is corroborated by data on morphological and cultural characteristics. SSCP analysis is generally useful for fragments of shorter lengths (300 bp or less). However, this study has shown that it can also be useful for fragments up to 600 bp. Using the appropriate control strains, SSCP can be applied in identifying isolates even up to species level.

Keywords: SSCP, *Aspergillus*, rDNA, ITS

HSD No. 10a

SEROTYPES OF DENGUE VIRUSES ISOLATED FROM PATIENTS ADMITTED AT ST. LUKE'S MEDICAL CENTER, QUEZON CITY IN THE YEAR 2001

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Dengue infection is a major health problem in the Philippines and it occurs every year all year round with peaks during the rainy season. The caus-

ative agent of the disease is the mosquito-borne dengue virus (family Flaviviridae) of which there are 4 serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. In the year 2001, more than 25,000 patients were admitted in hospitals all over the country. Serum samples from 461 patients were sent to the Research and Biotechnology Division (RBD) of St. Luke's Medical Center (SLMC) for laboratory confirmation of the disease through IgM-capture ELISA, a technique that detects the presence of IgM antibodies against dengue. The samples were also used as inoculum to infect the C6/36 mosquito cell line for virus isolation. Two hundred two (202) out of the 449 samples (45%) were found positive for the presence of flavivirus in the infected culture fluid by focus-formation assay. Reverse transcription polymerase chain reaction (RT-PCR) of the positive infected culture fluid showed that 146 samples were positive for dengue. The most prevalent serotype for 2001 was DEN-2 (112 samples), followed by DEN-1 (18 samples), DEN-3 (3 samples) and DEN-4 (2 samples). Some samples showed co-infections of more than one serotype: 6 samples were positive for both DEN-1 and DEN-2, 2 samples for DEN-1 and DEN-3, 2 samples for DEN-2 and DEN-3, and one sample for DEN-2 and DEN-4.

Keywords: dengue virus, flavivirus, IgM-capture ELISA, focus-formation assay, reverse transcription polymerase chain reaction

HSD No. 10b

PLASMID CONSTRUCTION AND POLYHISTIDINE-TAGGED DENGUE-2 VIRUS ENVELOPE PROTEIN EXPRESSION

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A more rapid and efficient site-specific recombination reaction, instead of the conventional restriction enzyme digestion and ligation protocol, has been employed in this experiment using the new Gateway™ Cloning Technology (Life Technologies). In this method, the previously sequenced SLMC 179 dengue-2 virus envelope gene was rescued from virus culture cDNA and modified

through high-fidelity PCR to generate and amplify a Gateway-compatible amplicon. Subsequent BP and LR recombination reactions produced entry and Pdest17-based expression constructs, respectively. BL21-S1 *E. coli* competent cells were then transformed with the plasmid construct. Expression of the recombinant amino terminal (His)6-tagged envelope protein was facilitated through induction with 0.3 M NaCl for 5 hours. Differential expression of total cellular protein and western blotting using mouse anti-6xHis monoclonal antibody verified the presence of the desired protein product. In forthcoming studies, this recombinant envelope protein will be purified by immobilized metal affinity chromatography and renatured to be used as an antigen for ELISA-based diagnosis of dengue virus and for the production of dengue-2 envelope-specific monoclonal antibodies. Furthermore, this could serve as a precursor in the development of candidate dengue sub-unit vaccine.

Keywords: recombination, dengue-2 virus envelope gene, high-fidelity PCR, expression, western blot

HSD No. 11

CHARACTERIZATION OF THE MANILA FAMILY OF MYCOBACTERIUM TUBERCULOSIS IN FILIPINO PATIENTS WITH PULMONARY TUBERCULOSIS

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Based on DNA fingerprinting, the population structure of *M. tuberculosis* has been found to vary from region to region. Notable among these are the Beijing-, Haarlem- and African Genotype families. Additional DNA technologies also confirm the family groupings of *M. tuberculosis* such as spoligotyping analysis, analysis of

pseudogene *acyR*, polymorphic GC risk sequences (PGRS) Restriction Fragment Length Polymorphism and variable numbers of tandem repeats (VNTR) typing. No study has yet been done to identify the genotype family of M. tuberculosis isolates in the Philippines.

Forty *M. tuberculosis* isolates were analyzed from individual pulmonary TB cases included in a regional prevalence survey of tuberculosis conducted by the University of the Philippines College of Medicine and the Research Institute for Tropical Medicine from December 1995 to August 1996. These *M. tuberculosis* isolates were heat-killed at 80 C in TE buffer and then transported to the University of Hawaii in Honolulu for DNA analysis employing different techniques.

Comparison of the IS6110 RFLP and the spoligopatterns were performed using the Compar software at the RIVM in Bilthoven, the Netherlands. The results were compared with the international database of IS6110 RFLP patterns consisting of 5906 patterns from 30 countries and the international database on spoligopatterns consisting of 3575 patterns from 55 countries. Comparisons were done using the Dice coefficient for calculation of similarities.

The IS6110 RFLP pattern of the M. tuberculosis isolates from the Philippines exhibited a high degree of similarity, yet none of them were identical. 38 (95% of the 40 isolates showed a similarity of 80% or greater between the patterns. We designated these 38 isolates the "Manila Family."

It is clear that the high frequency of occurrence, restricted geographical range and similarities of the IS6110 RFLP patterns of the Philippine isolates of *M. tuberculosis* indicate a unique family of M. tuberculosis. These findings may be valuable in providing the international database and reference for future studies that will identify geographic distribution of these *M. tuberculosis* isolates as well as studies in identifying modes of transmission among high risk groups that may impact on public health policy and programs on TB Control.

Keywords: DNA fingerprinting, Restriction Fragment Length Polymorphism (RFLP), Spoligotyping, *Mycobacterium tuberculosis*

HSD No. 12

HCV GENOTYPES IN THE PHILIPPINE POPULATION

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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. Patients undergoing hemodialysis and blood transfusion, renal transplant patients and blood donors found to have HCV infection by second generation EIA are included in the study. RNA from serum is extracted and a reverse transcription PCR is performed using nested primers from the highly conserved 5' non-coding region of the HCV genome. Samples positive for HCV RNA are genotyped by Restriction Fragment Length Polymorphism (RFLP). Out of the 99 serum samples reactive with anti-HCV by ELISA, 52 were confirmed positive by PCR. Of the 52 samples, 34 were genotype 1a, 9 genotype 1b, 3 genotype 2a, genotype 2b and 1 genotype 4 (Simmond's classification).

Keywords: reverse transcription PCR, Hepatitis C virus, restriction fragment length polymorphism, ELISA

HSD No. 13

FIVE-YEAR ANTIBIOTIC SENSITIVITY PROFILE OF LOWER RESPIRATORY TRACT PATHOGENS FROM HOSPITALIZED PATIENTS

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One thousand two hundred twelve (1,212) lower respiratory tract pathogens commonly isolated from hospitalized cases were collected over a period of five years (1998-2002) from three Metro Manila hospitals and tested for susceptibility to various antibiotics to determine resistance trends. Specimen included sputa, endotracheal aspirates, and bronchial washings. The isolates were identified and tested for susceptibility using the Vitek 60 (Biomerrieux). Four hundred sixty-six (38.4%) were identified as *Klebsiella* spp, 480 (39.6%) as *Pseudomonas* spp, 166 (13.4%) as *Enterobacter,* and 100 (8.2%) as *E. coli*. The study employed nine antibiotics representing 4 classes: beta-lactams (ampicillin-AMP, cefazolin-CZ, cefuroxime-ROX) and beta-lactam/b-lactamase-inhibitor (ticarcillin/clavulanic acid- TCC) combination, aminoglycosides (gentamicin- GM, tobramycin- TOB), fluoroquinolones (ciprofloxacin- CIP), and a sulfonamide-trimnthoprim combination (trimethoprim/sulfamethoxazole- SXT).

Results indicate that the beta-lactams tested only CZ remains effective to *E.coli* and *Klebsiella*. Ampicillin is exhibiting an increasing efficacy against *E. coli* over the last 3years. *Enterobacter* spp has shown a consistently increasing resistance to ROX, while *Pseudomonas* spp showed resistance to all the beta-lactams tested. Of the two drug combinations tested, TCC exhibited a consistently better activity over SXT against all the isolates tested. The aminoglycosides have been effective against the isolates over the 5-year period. An upward trend has been noted for ciprofloxacin against *E. coli* and *Pseudomonas* spp, but a gradual decreasing sensitivity has been indicated for *Klebsiella*.

Although sampling size is limited, the study reveals an insight to the responses of the top pathogens commonly isolated from hospitals, and results indicate that the organisms are developing resistance to antibiotics. It is recommended, therefore, that antibiotic prescription be always preceded by susceptibility tests, and that appropriate guidelines be followed strictly by both patients and medical practitioners to control the emergence of antimicrobial resistance.

Keywords: antibiotic sensitivity, lower respiratory tract, pathogens

HSD No. 14

CLARITHROMYCIN RESISTANCE IN HELICOBACTER PYLORI ISOLATED FROM FILIPINO PATIENTS: DETECTION BY PCR-RFLP

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Helicobacter pylori is a microaerophilic bacterium known to cause gastritis, peptic ulcer, and gastric carcinoma. A major problem in *H. pylori* treatment is resistance to clarithromycin, which is a component of the widely used therapy in the eradication of the organism. Resistance of *H. pylori* to clarithromycin is associated with point mutations in the 23S rRNA gene, mainly at positions 2142 and 2143. In this study, A2143G mutation in the 23S rRNA gene was examined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) in five clarithromycin resistant, one intermediate, and three sensitive *H. pylori* isolates from Filipino patients. Epsilometer test was used to determine clarithromycin susceptibility of the isolates. PCR-RFLP using primers CLA 18 and CLA 21, as well as restriction enzyme *Bsa*I was employed. The clarithromycin sensitive and intermediate isolates included in the study were wild type by PCR-RFLP. In the RFLP patterns obtained, none of the resistant isolates (5/5) exhibited A2143G mutation commonly associated with clarithromycin resistance.

Keywords: *Helicobacter pylori*, 23S rRNA gene, clarithromycin resistance, point mutation

HSD No. 15

*vac*A, *cag*A, AND *ice*A, GENES: CORRELATION WITH GASTRODUODENAL DISEASES IN *Helicobacter pylori* INFECTION

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H. pylori infection is strongly associated with chronic gastritis, peptic ulcer diseases and in the subsequent development of gastric adenocarcinoma. Three genes, the vacuolating cytotoxin (*vac*A), the pathogenicity island (*cag*PAI) gene, and the *ice*A (induced by contact with epithelium) are at present the main well established virulence factors of *Helicobacter pylori*. Their presence is among the many other bacterial factors responsible for the successful colonization and establishment of persistent infections.

This study aimed to assess which of these virulent factors could be correlated with the clinical outcome of gastroduodenal diseases. We studied 64 gastric biopsy samples taken from 16 cases of duodenal ulcer, 16 cases of gastric ulcer and 32 cases of gastritis, for the presence of *cag*A, *vac*A, and *ice*A genes by Polymerase Chain Reaction (PCR) detection using specific primers. All 64 samples were completely genotyped by PCR. The s1a m1 genotype was most common in gastritis (62.5%) and gastric ulcer (37.5%) and the s1a m2 genotype occurred in 31.5% of duodenal ulcer. The *cag*A gene predominates in duodenal ulcer (62.5%), followed by gastritis(59.37%) and gastric ulcer (56.25%). The *ice*A2-1 gene was detectable in 50% of duodenal ulcer, and 43.75% of gastritis and gastric ulcer, while the *ice*A1 gene was present in 43.75% of gastric ulcer, 37.5% of duodenal ulcer and in 9.38% of gastritis. Of the three genes, the *vac*A genotypes were better correlated with the clinical outcome of gastroduodenal disease

Keywords: *Helicobacter pylori*, *vac*A, *cag*A, *ice*A, Gastroduodenal diseases, PCR

HSD No. 16

INSULIN AND RETINOIC ACID INHIBIT BRANCHING MORPHOGENESIS OF EMBRYONIC LUNG BUDS IN ORGAN CULTURE

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The embryonic lung has been shown to develop through epithelial-mesenchymal interaction undergoing dichotomous branching to form an intricate tree-like structure. Various studies in vivo and in vitro have elucidated the role of several genes involved in lung function and development and these have helped define the mechanisms involved in branching morphogenesis. Knowing important mechanisms in lung development can help create better clinical methods for the cure of lung diseases as well as in devising gene therapeutic strategies for ameliorating lung injury. A better understanding of lung development and mechanism would help provide a more specific approach to individualized treatment of respiratory diseases. In this study, the effects of various substances on lung branching morphogenesis *in vitro* was investigated. We made use of ICR mouse embryo (11.5 dpc) lung bud explants collected in Leibovitz L15 medium and cultured for six days on top of a collagen-coated Nucleopore filter. We investigated the development of the bronchial tree in different culture media such as Leibovitz L15 which does not require CO_2 during culture, Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (D10), D10 supplemented with insulin-transferrin-selenious acid (ITS^+), and D10 added with retinoic acid (RA). Lung morphogenesis was captured daily using an Olympus digital camera and images were normalized and processed using Adobe Photoshop 5.0. Explants were observed with respect to the complexity of branching which was analyzed using fractal analysis. Among the results obtained, the effect of insulin in the cultures was most notable. Results revealed that when ITS^+ was added to the cultures, a more proximal differentiation pattern was obtained as opposed to the normally dichotomously branched pattern which increases in complexity as the lung develops distally. The same effect was observed with lung cultures in D10+RA. These results suggest that insulin and RA inhibit distal lung branching morphogenesis.

Keywords: lung branching, insulin, retinoic acid, lung development

HSD No. 17

PCR-DETECTION OF CEA mRNA IN REGIONAL LYMPH NODES OF PATIENTS WITH COLORECTAL CANCER

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The prognostic potential of reverse transcriptase-polymerase chain reaction (RT-PCR) on carcinoembryonic antigen (CEA) determination was investigated and compared with the histopathological method for lymph node status determination in colorectal cancer.

Detection of CEA by RT-PCR was performed on lymph nodes taken from patients with colorectal cancer and benign colorectal diseases. Lymph nodes were resected from pericolic and peritumoral regions and total RNA was extracted from each note separately. Primers specific for the CEA gene were used and the presence of CEA messenger ribonucleic acid (mRNA) in lymph node samples was considered evidence of metastasis.

Thirty-five or 37% of 94 mRNA saples obtained from histologically negative lymph nodes were found positive for CEA by RT-PCR. Lymph nodes from a patient with benign colorectal disease exhibited no CEA mRNA. Overall, 43 (44%) of the total 102 lymph nodes were positive by RT-PCR detection compared with only eight (7%) lymph nodes positive for micrometastases by histopathological analysis.

This study shows that RT-PCR of CEA mRNA in patients with colorectal cancer may provide additive value to histopathological analysis in detecting lymph node micrometastasis and predicting recurrence, especially when micrometastasis spreads out separately from the main tumor to distant lymph nodes.

Keywords: CEA, carcinoembryonic antigen, mRNA, messenger ribonucleic acid, colorectal cancer, pericolic, peritumoral

HSD No. 18

MULTI-LEVEL DETECTION OF HEMOGLOBINOPATHY AMONG BLOOD DONORS AT ST. LUKE'S MEDICAL CENTER

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Hemoglobinopathies are diseases resulting from hereditary abnormalities of the hemoglobin chains. The abnormality (mutation) may alter globin protein, its synthesis or globin development switching. This results in either complete absence or defective synthesis of different hemoglobin types. Thalassemia, a disease resulting from an imbalance of globin-chain synthesis belongs to this group. The pathophysiology of this disease lies in the resulting imbalance (ratio) between the alpha (a) and beta (b) globin gene synthesis. The chain that is produced at the normal rate is in relative excess. In the absence of a complementary chain with which to form a tetramer, the excess normal eventually precipitates in the cell, damaging the membrane and leading to premature blood cell destruction. Clinical manifestation of the disease ranges from mild anemia to hydrops fetalis depending on the degree of mutation.

A multi-level detection of the disorder was conducted on blood donors of St. Luke's Medical Center. The study aims to characterize the thalassemia mutations at the cellular and molecular level and to determine the frequency of hemoglobinopathies among blood donors.

Blood samples were initially screened using complete blood count with red cell indices specifically Mean Corpuscular Volume (MCV) and serum ferritin. Of the 1,468 samples, 70 samples were included based on the inclusion criteria of MCV less than 80 fl and serum ferritin of greater than 12 g/dl. The presence and the amount of the different hemoglobin types were densitometrically measured and analyzed. Four samples with an abnormally high amount of HbA_2 greater than 3%, were observed. Samples with MCV less than 80 fl were consequently run in PCR using primers specific for alpha thalassemia.

Keywords: hemoglobinopathy, thalassemia, mean corpuscular volume, serum, ferritin, polymerase chain reaction (PCR)

HSD No. 19

LOW PROPORTION OF DYSTROPHIN GENE DELETIONS AMONG FILIPINO DUCHENNE AND BECKER MUSCULAR DYSTROPHY PATIENTS

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Duchenne muscular dystrophy (DMD) is an X-linked progressive neuromuscular disorder affecting 1 in 3500 male livebirths. It has a milder allelic form, Becker muscular dystrophy (BMD) which occurs in 1 in 20,000. Both are caused by mutations in the dystrophin gene on the X chromosome. In DMD, affected individuals have little or no functional dystrophin while those with BMD may have a partially functional dystrophin giving rise to its milder clinical manifestations.

Mutations that cause DMD/BMD are deletions of one or more exons of the dystrophin gene, duplications of the exons or point mutations. The proportion of deletions among mutant dystrophin alleles in North American and European studies is 55-65 % [Baumbach et al.,1989; Koenig et al.,1987; Forrest et al.,1988]. Shomrat et al. [1994] described a lower proportion (37%) of dystrophin gene deletions among Israeli DMD and BMD patients. Other Asian populations have likewise shown a lower proportion of deletions among mutant dystrophin: 40-43% in Japan [Sugino et al.,1989; Kitoh et al.,1992]; 45-50% in Chinese populations [Soong et al.,1991; Lau et al., 1992]. A racial difference in the proportion of deletions may exist

We examined DNA samples of 41 unrelated Filipino patients diagnosed with DMD and BMD. Of these patients, 15 have already been included in a previous report [Cutiongco et al .,1995]; this is an extension of that study. Deletions were detected using multiplex DNA amplification procedure which permits the rapid identification of 80-90% of all dystrophin gene deletions [Chamberlain et al .,1988].

Our results show that only 11 out of the 41 patients (26.82%) have deletions in at least one of the 23 exons of the dystrophin gene examined. This proportion of dystrophin gene deletion is the lowest reported so far compared to other populations. This low proportion makes diagnosis using the current PCR techniques particularly difficult. In addition, we found that the deletions among Filipino DMD/BMD patients were more common in the 5' end (63.63%) than in the central rod domain (36.36%) of the dystrophin gene. The findings suggest the presence of genetic variability among DMD/BMD patients in different populations.

Keywords: Duchenne Muscular Dystrophy, DMD, Becker Muscular Dystrophy, BMD, dystrophin gene, X-linked

HSD No. 20

MUTATIONS OF THE STEROID 21-HYDROXYLASE GENE AMONG FILIPINO PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

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Congenital Adrenal Hyperplasia (CAH), an autosomal recessive disorder, is due to defective enzymes involved in adrenal steroidogenesis. Phenotypic manifestations are variable depending on the effects produced by the deficient hormones and by the excess production of steroids unaffected by the enzymatic block. The worldwide incidence of CAH is 1 in 15,000 with ethnic and racial variability. The incidence in France, Italy, Scotland, New Zealand and Japan ranges from 1 in 10,000 to 1 in 23,000. Among Filipinos, the crude incidence of CAH is 1 in 6,747 (Philippine Newborn Screening Update, 2002), which is higher than what is reported in most populations. More than 90% of all cases result from a 21 hydroxylase (cytochrome P450c21) deficiency involving two 21 hydroxylase genes CYP21, the active gene and CYP21P, a pseudogene. Studies have shown that mutations result from unequal crossover during meiosis which leads to complete deletion of the gene, gene conversion events or to point mutations. Major-

ity of these studies have demonstrated differences in the frequency of several gene mutations. Using a previously described method (Lee et al, 1996) of combined differential Polymerase Chain Reaction (PCR) and Amplification Created Restriction Site (ACRS) approach, direct probing for the presence of known mutations in exon 1,3,4,6,7,8 and intron 2 of the CYP21 and CYP21P genes among Filipino patients with CAH was performed. A total of 12 unrelated CAH patients were examined. One of the twelve cases (8%) demonstrated two different mutations in her CYP21 gene; in ten of the twelve cases (83%) only one type of mutation was detected. Majority of these cases had the mutation at nucleotide 656 of intron 2, a premature splicing error. The determination of the most frequent alleles in our population will facilitate rapid screening for detection of mutations in the 21 hydroxylase gene. Establishment of a definitive diagnosis can be also made available which are important in the management and counseling of Filipino CAH cases.

Keywords: congenital adrenal hyperplasia, CAH, CYP21 gene, CYP21P gene, Amplification Created Restriction Site (ACRS)

HSD No. 21
USE OF FLUORESCENCE IN SITU HYBRIDIZATION (FISH) IN THE DIAGNOSIS OF FILIPINO PATIENTS WITH PRADER-WILLI SYNDROME

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Prader-Willi syndrome, or PWS, is a complex disorder, involving multiple systems with many manifestations including infantile hypotonia, developmental delay and mental retardation, behavior problems, obesity, characteristic facial features, hypothalamic hypogonadism, and short stature. The cause of PWS is the absence of normal active paternal genes in the proximal region of chromosome 15q. There are three different types of mutations on chromosome 15 which may

lead to absent normal paternal genes in this region: (1)paternal interstitial deletion, (2)maternal uniparental disomy (UPD), or (3) mutation or abnormality in the imprinting center. Deletions account for 70-75% of the cases, 25-28% have maternal UPD. and <2% have defects in the imprinting center. High resolution G-banding of chromosome 15 has been shown to be an unreliable method to diagnose the deletions while fluorescence in situ hybridization (FISH) with specific DNA probes has proven to be more sensitive in detecting the deletions. The recent availability of this genetic test locally has given us the chance to confirm suspected PWS cases and to characterize the mutations in the Filipino population. Fourteen unrelated Filipino PWS patients were included in the study. Cytogenetic analysis was normal for all patients but the FISH technique detected deletions in 50% of the subjects. The confirmation of the diagnosis will now allow the determination of the recurrence risk for the families concerned.

Key words: Prader-Willi Syndrome, PWS, fluorescence-in-situ hybridization, FISH, microdeletion

HSD No. 22

TWO FILIPINO CASES WITH RARE AUTOSOMAL TRISOMIES

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Trisomies for autosomes other than chromosomes 21, 18, and 13 are noted to have severe consequences and inevitably result to fetal death *in utero*. There are rare instances wherein these other autosomal trisomies survive the neonatal period. We report two such cases. The first is a case of a 1-month old male born premature to a non-consanguineous Filipino couple. This case presented with intrauterine growth retardation, microcephaly, hypertelorism, epicanthic folds, midface hypoplasia, low set ears, multiple ventricular septal

defects and a hypoplastic 5th distal phalanx. The diagnosis of Trisomy 22 was confirmed in all cells examined by chromosomal analysis (G-banding). The second case is a 3-month old female born term to non-consanguineous Filipino parents. She presented with a high arched palate, low set ears, bifrontal hair whorls, unequal palpebral fissures, hypertelorism, limited movement of the left eye, overlapping fingers on the right hand (5th digit over the 4th digit, 2nd digit over the 3rd digit), large flat nipples with a hypopigmented central area of the areola, a sacral dimple, and a wide gap between the first and second toes. Chest radiographs showed eleven pairs of ribs. Chromosomal analysis by G-banding confirmed the diagnosis of Trisomy 11. To our knowledge, there are no other case reports of trisomy 11 in literature. Mosaicism is not discounted as a possible cause for the prolonged survival of our patient. These cases add to the world literature on autosomal trisomies in liveborns.

Keywords: autosomal trisomy, trisomy 11, trisomy 22, multiple congenital anomalies, chromosome 11, chromosome 22

HSD No. 23

INITIAL VERSUS CONFIRMATORY THYROID STIMULATING HORMONE (TSH) LEVELS: IS THERE A CORRELATION?

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Newborn screening for congenital hypothyroidism (CH) in the Philippines began in 1996. The screening method used is the fluoroimmunometric assay of the thyroid stimulating hormone (TSH) from the blood impregnated in the Guthrie card. In the past five years (June 1996-September 2001), 176,548 newborns have been screened. Of these, 237 had elevated TSH levels, and approximately 51 (22%) are confirmed to have CH. 146 (61%) had normal TSH levels on confirmatory testing; five (2%) expired; 25 newborns (11%) are lost to follow-up, while 10 (4%) are currently being recalled. 33 out of 51 (65%) CH patients are female. Only 38 of the 51 patient charts were available for data analysis. Thirteen out of 51 CH patients were lost to follow-up after confirmation of the disorder. The mean age at which levothyroxine medication was initiated is 1 ½ months at a modal dosage of