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The First Joint Congress of the College of Pathologists and Malaysian Institute of Medical Laboratory Sciences was held at the Crown Princess Hotel, Kuala Lumpur from 16th to 18th December 2003. Abstracts of free papers presented follow:

Oral presentations:

1. **A Rapid Chromatographic Immunoassay for the Diagnosis of Syphilis Infection**

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The venereal disease, syphilis is caused by Treponema pallidum, a spirochete bacterium. It is usually transmitted through sexual intercourse, vertical transmission from infected mother to the foetus, and through blood transfusion. VDRL and RPR are non-treponemal serology tests widely used for screening purposes. TPHA, TPPA, FTA-ABS or ELISA are Treponemal tests used in confirming syphilitic infection. The Acon syphilis (Acon Laboratory USA), is a one step chromatographic immunoassay for detecting Treponema Pallidum IgM and IgG in both serum and plasma. The objective of this study is to evaluate the performance and the usefulness of Acon syphilis one step ultra (Acon Laboratory USA) in the diagnosis of syphilis infection. A total of 520 samples were used in this study. All the samples were tested with Rapid Plasma Reagin (RPR Macro-vue BD), Treponema pallidum particle agglutination (TPPA Fujirebio Inc Japan) and Acon syphilis one step ultra. Compared to TPPA, the Acon syphilis one-step ultra Test exhibits a relative sensitivity of 99.64% (156/157). The Acon syphilis correctly identified all 363 negative samples exhibiting a relative specificity of 100%. Based on the results of this evaluation, the sensitivity and the specificity of Acon syphilis one step ultra syphilis test is comparable to TPPA used for the detection of Treponema pallidum antibodies. This assay is easy to perform and requires no sophisticated and expensive equipment.

2. **Detection and Identification of Legionellae using Duplex PCR targeting the 16s rRNA and icm Genes**

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The purpose of this study was to design a rapid detection method for the identification of Legionella pneumophila, the causative agent for Legionnaires' Disease, using Polymerase Chain Reaction to amplify portions of its 16s rRNA gene and icm WXYZ virulence genes. The study was carried out using both L. pneumophila serogroup (1,4,5,6,7,12,13) controls and local isolates (both L. pneumophila and non-pneumophila) from cooling towers. Collected water samples were filtered by negative pressure through a polycarbonate membrane, which was subsequently washed in sterile water to dislodge trapped organisms. The water was then centrifuged, and the pellets were subjected to 15 minutes of acid-buffer treatment before being plated on Buffered Charcoal Yeast Extract Agar (BCYEA) and incubated at 37°C for 7-14 days. Colonies which appeared after 3-5 days of incubation, and were grayish-white in colour with round edges and shiny appearance were presumed to be Legionella. DNA was extracted from these cells using lysis buffer-boiling method, followed by DNAzol treatment. Amplification of the DNA was carried out in two sets of duplex reactions, using primers targeting (1) the 16S rRNA and icm WX genes, and (2) the 16S rRNA and icm YZ genes; as well as a third reaction with a set of primers targeting the entire icm XYZ region. The amplicons were electrophoresced and visualized under a UV transilluminator. All L. pneumophila control serogroups and legionella species from cooling towers were found to display a band

corresponding to the 16S rRNA targeted sequence. All L. pneumophila controls and isolates displayed another band corresponding to the targeted icm WX sequence. L. pneumophila serogroup 1 controls and isolates showed bands corresponding to all the targeted icm WX, YZ and XYZ sequences. Some of the L. pneumophila serogroups 2-14 isolates failed to display the YZ band; however, amplification with the icm XYZ primers confirmed the presence of this gene in some of these isolates, suggesting variations in the YZ sequence among serogroups. Legionella species other than L. pneumophila were only positive in the 16S rRNA PCR and had none of the icm WXYZ genes. The method described here has proved to be effective in the rapid detection of Legionella organisms in water samples. By using the first duplex reaction it is possible to differentiate between L. pneumophila and Legionella non-pneumophila organisms; the second duplex reaction and third PCR allow detection of serogroups 1-14. Confirmation of individual L. pneumophila serogroups can be obtained by DNA sequencing, when required.

3. Traditional PCR versus Real-Time PCR Detection of Male-Specific DNA Coliphages

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Polymerase Chain Reaction (PCR) is an advanced molecular technique in clinical diagnostic and environmental microbiology. The PCR technique allows multiple copies of a DNA molecule to be generated by *in vitro* enzymatic amplification of a target DNA sequence. The real-time PCR technology was developed recently to allow real time monitoring on the amplification process and quantifying the amplicons in each cycle. This method is preferred over traditional PCR because it is rapid, sensitive and no post-PCR processing is required. The aim of this project is to compare the traditional PCR and real-time PCR for the detection of male-specific DNA coliphages. The detection range of the traditional PCR assay for 10-fold dilutions of M13 coliphages was 10^{-3} to $\geq 10^{2}$ plaque forming units per reaction mixture, whereas the detection range of real-time PCR is between 10^{-2} and $\geq 10^{6}$ plaque forming units per reaction mixture, with C(t) values ranging from 18 to 37. In this study, we showed that the sensitivity (R^2) of the traditional PCR and real-time PCR is 0.9506 and 0.8939 respectively. However, the efficiency of the traditional PCR and real-time PCR is 0.9 (slope = 20.656) and 2.52 (slope = -2.4911) respectively. In summary, we have shown that the real-time PCR assay is a rapid, sensitive, specific, reproducible and simple method of quantitative detection of M13 FDNA coliphages. On the other hand, traditional PCR allows only qualitative detection.

4. Serological Correlation between Adeno-Associated Virus (AAV) and Human Papilloma-Virus (HPV) in Cervical Cancer Patients

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Introduction: Adeno-associated virus (AAV) utilizes the functions of helper viruses such as the adenovirus or human papillomavirus, or genotoxic agents for survival in their host cells. The AAV-2 and AAV-3 serotypes are frequently isolated from human clinical specimens and are strongly homologous. Approximately 80% of the adult Caucasian populations have been reported to be seropositive for the AAV. Reports have also demonstrated that the AAV is negatively associated with cervical cancer, an observation attributed to the suppressive activity of the AAV on the Human Papillomavirus (HPV).
Objectives of Study: (1) To determine the serological status of AAV in cervical cancer patients and (2) to evaluate whether there is any serological correlation between AAV and HPV in cervical cancer.
Materials and Method: The antibodies for both AAV and HPV were determined using enzyme-linked immunosorbent assays (ELISAs) designed in-house. Briefly, these assays consisted of coating

purified AAV or HPV "Virus-like Particles" (produced using cell culture technology) onto microtiter plates for use as capture antigens for the respective antibodies. A preliminary serological survey of the normal adult population (males and females) was conducted to provide baseline information on the local population. The study group was patients with diagnosis of cervical cancer based on cytological and/or histological evidence. For evaluation of the AAV serological prevalence in cervical cancer patients, age-matched, non-pregnant female adults were used as controls.

Results and Conclusions: A total of 417 healthy adults were tested, of whom 61% were seropositive for AAV-2 while 50% were seropositive for AAV-3. The observed AAV-2 seroprevalence was higher in males compared to females (68% versus 51%), while the reverse was true for the AAV-3 seroprevalence (64% for females versus 40% for males). The seroprevalence of AAV was statistically significantly higher in the married population (X^2 =14.44, $p<0.001$) suggesting the possibility of virus transmission through sexual contact. A negative association was found between AAV-3 seroprevalence and cervical cancer when compared to normal non-pregnant women. This observation suggests a possible protective role of AAV-3 against cervical cancer. However, there was no significant difference in the seroprevalence of AAV between HPV positive and HPV negative cervical cancer patients. This suggests that the HPV may not have a major role as helper virus for AAV. Nevertheless, the observation that AAV seropositivity is twice as frequent in HPV negative cancer patients than in HPV positive patients may be supportive of a suppressive effect of AAV on HPV.

5. Antiretroviral Drug Resistance Testing in Adults Infected with Human Immunodeficiency Virus Type 1

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Objective: To study the prevalence of HIV-1 drug resistance mutations in drug-naïve and pre-treated adult patients in University Malaya Medical Center (UMMC).

Methodology: Plasma viral RNA was extracted from 43 HIV-1 seropositive patients, among whom 24 were drug-naïve and 19 were pre-treated with antiretroviral. Reverse transcriptase (RT) and protease (PR) genomic regions were amplified via nested-PCR, sequenced and analyzed for drug resistance mutations.

Results: Among drug-naïve patients, 19 and 24 patients were successfully sequenced for the RT and PR genes, respectively. Primary RT mutation Y181C was found in one of the patients (1/19 = 5%) while M46I of PR gene was observed in one patient (1/24 = 4%). Secondary RT gene mutation K43E was seen in 68% of the patients, and mutations I13V, K20R, E35D and M36I in the PR gene were present at 67%, 33%, 67% and 70%, respectively. In antiretroviral-experienced patients, 13 and 19 patients were successfully sequenced for the RT and PR genes, respectively. In the RT gene, primary mutations conferring to NNRTI resistance, K103N (38%), Y181C (15%), Y188L (23%) and G190A (8%) were observed. M184V substitution was also present (23%). Secondary mutations K43E and I178M were both found at 62%. I13V, E35D and M36I minor mutations were also observed in PR gene (89%, 95% and 89%). It was also noted that 89% of treated patients harbored the I13V + E35D + M36I mutation, as compared to 42% of drug –naïve patients

Conclusion: The prevalence of drug-resistant HIV-1 circulating among drug-naïve patients is low but major mutations conferring NNRTI resistance is high in treated patients. Continued surveillance is required to determine an accurate trend in this prevalence. Expert interpretation of genotypic test results is required, given the complexities of drug regimens and mutational interactions

6. **Impact of Changing Technologies on Alloantibody Identification Patterns in a Teaching Hospital**

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Continued evolution has been seen over the past decade in the performance of pretransfusion testing with continued improvements in technologies for antibody screening. A good antibody screening technique should be sensitive to all clinically relevant antibodies but not overly sensitive leading to high false positive rates and the detection of clinically irrelevant antibodies. Over the past 5 years, we have changed our antibody screening techniques from tubes to column agglutination using a gel matrix (DiaMed) and to column agglutination using microbeads (BioVue). We report here our experience with the pattern of antibodies identified at our centre after screening using tubes (1998), DiaMed polyspecific AHG gel cards (1999 – 2000) and BioVue anti-IgG AHG cassettes (2002 – 2003). There was a transition between the use of DiaMed gel cards and BioVue cassettes in 2001, and thus data from that year is not reported. A total of 664, 2595 and 599 samples were investigated for antibodies after being found positive on screening with tube, DiaMed cards and BioVue cassettes respectively. 58.1% of the positive antibody screens using the tube test after exclusion of the room temperature antibodies had no detectable antibodies when investigated using the panel of antibody identification cells. This compares with 17.4% and 17.9% with DiaMed cards and BioVue cassettes respectively. 16.4% of the samples found positive on screening with BioVue cassettes were found to have a pattern on antibody identification panel with no definite assignable specificity. Only 4.7% of antibody screens positive with DiaMed had no assignable antibodies identified. Anti-E was identified in 9.8%, 2.8% and 3.3% of the samples positive on screening with BioVue, DiaMed and tube test. Anti-M was observed in 2.8% of sample screened with BioVue as compared to 0.3% with DiaMed. Lewis antibodies were the most common antibody identified with 23.9% of samples positive during the period 1999 - 2000 and 18.5% of samples positive during 2002 - 2003 having anti-Le^a. However, the detection of anti-Le antibodies during the period 1999 – 2000 was attributed to the inclusion of an additional room temperature tube screening rather than the use of the DiaMed card, which was generally insensitive to anti-Le antibodies. Anti-Mia was also more commonly detected during this period. No significant differences were observed with other recognized antibodies. In conclusion, tube tests have a high false positive rate compared to either of the column agglutination technologies. The BioVue cassette however appears to be more sensitive to the presence of anti-M and anti-E. Despite using a monospecific anti-IgG AHG cassette anti-Lewis antibodies were still commonly identified using the BioVue cassette. In addition, the BioVue appears to be more sensitive to non-specific antibodies with no assignable specificities when tested against a panel of identification cells.

7. **High Resolution Cytogenetics – Results of Analysis of a Neonate with Multiple Deformities and an Adult Female with Reproductive Failure**

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The principal aim of cytogenetic services is to provide information to individuals at risk of genetic disease or of chromosomal anomalies. Post-natal chromosome analysis should be considered for subjects with the following risk factors :

- Congenital anomalies in neonates
- Developmental delay in childhood
- Delayed growth and sexual abnormalities in adolescences
- Infertility / subfertility
- Recurrent miscarriages
- Family history of genetic disease

The Cytogenetics Laboratory in the University Malaya Medical Centre (UMMC) provides high-

resolution cytogenetic analysis using modified protocols. Synchronization of cell cycle is carried out to maximize cell harvesting at the pro-metaphase stage. Cell cycle is blocked using methotrexate (MTX) and released using thymidine. Mitosis is arrested with a short colchicine treatment. G banding is performed using Wright stain, which has been found to be superior to the standard Giemsa stain as it was able to provide higher banding resolution even on short chromosomes. We report the findings of analysis on a stillborn infant with multiple foetal abnormalities and an adult female with history of frequent miscarriages. Cord blood was collected from the stillborn baby for karyotyping. A total of twenty-nine chromosome spreads were analysed and the results showed a complex karyotype: 47,XX,+10(19) / 47,XX,+22(6) / 48,XX,+10,+22(2) / 46,XX(2). The second case involved a woman with four previous miscarriages and no successive pregnancy in five years. Her karyotype was found to be 46,XX,inv(9) with a pericentric inversion. The chromosome abnormality was also detected in the proband's sister but not in the brother. The sister had a terminated pregnancy due to trauma but she has no history of sub-fertility. Pericentric inversion of the chromosome 9 is a common occurrence and has been considered as a normal variation. The incidence is about 1% to 1.65% in the general population. However, reports have also associated this abnormality with sub-fertility and recurrent abortions.

8. P53 Gene Mutations in Malignant Lymphoma

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Background: Tumour suppressor gene, p53 is a common target in carcinogenesis, reported to be functionally inactive and altered in 70% of human cancers. Although p53 mutations are less commonly present in haematological malignancies when compared to other solid tumours, nonetheless they have been reported in histological transformation of follicular lymphoma (FL) to higher grades tumours.
Aim: This study aims to investigate the pattern of p53 alterations on paraffin embedded tissue samples of FL and diffuse large B-cell lymphoma (DLBCL).
Material and methods: Surgical samples of FL from 7 patients (17 sequential biopsies) and 62 cases of DLBCL were retrieved for the study of p53 gene expression using immunohistochemical stain, and gene status by PCR-SSCP.
Results: p53 is distinctly over-expressed in the 5 transformed higher grade biopsies, and all except one showed electrophoretic mobility shift in PCR-SSCP. Sequencing analysis confirmed p53 mutation in 3 out of 4 of these cases (75%), whereas some other studies reported lower frequency of 25-30%. Lower grade tumours did not over-express p53, and did not demonstrate band shift nor reveal mutations. Over-expression of P53 protein was present in 48% of DLBCL, but only 9 cases had mutation of p53.
Conclusion: We demonstrated the feasibility of adopting PCR-SSCP for screening of p53 mutations in archival tissue samples, and there is a strong correlation of p53 gene over-expression and mutation events in high grade transformed of FL. However, there was no significant association between over-expression of P53 with mutations of p53 in DLBCL, concurring with previous reports.

9. An Immunohistochemical Study of the Expression of Thyroid Transcription Factor-1 in Nasopharyngeal Carcinoma

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Thyroid Transcription Factor–1 (TTF-1) is a 38.6kDa protein which controls morphogenesis and gene expression in the thyroid and lung. Immunopositivity for TTF-1 is limited to the epithelial cell nuclei. In this study we investigated the immunostaining characteristic for TTF-1 and its significance in nasopharyngeal carcinoma (NPC). An immunohistochemical (IHC) study using monoclonal TTF-1 antibody was performed on 35 tissue blocks of nasopharyngeal carcinoma

in 31 Malaysian patients. The diagnosis in each case was first reconfirmed on haematoxylin and eosin (H&E) sections. Then two sets of unstained sections were prepared from each paraffin block of the respective tumour tissue specimens. Each set of unstained sections was stained with monoclonal TTF-1 antibody via Streptavidin–biotin–peroxidase technique, but utilizing different types of buffer solutions, i.e. citrate buffer at pH6 (CB6) and ethylenediamine tetraacetate at pH8 (EDTA8), in the antigen retrieval process. The results showed that positive nuclear staining was detected more frequently in tumour sections processed using EDTA8 in the antigen retrieval process than CB6. However, although the IHC method using EDTA8 appeared to be more sensitive, all the sections processed using EDTA8 showed some degree of non–specific background staining. It was also revealed that the tumour cells in NPC which showed the strongest positive nuclear staining were invariably of the spindle cell type, the presence of which denotes poorer prognosis when compared with other tumour cell types in NPC. The overall results suggested that TTF-1 immunohistochemistry might be of prognostic significance in NPC, but different buffer solutions used in the antigen retrieval step affected its sensitivity and specificity.

10. **Chediak Higashi Syndrome: A Case Report**

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Chediak Higashi Syndrome (CHS) is a rare inherited disorder, which usually presents in early childhood with recurrent infection due to poor resistance. The natural course of this disorder is death at a young age due to infection or haemorrhage because of progressive leucopenia and thrombocytopenia.
A 5-month-old Chinese infant was referred to University Malaya Medical Centre for suspected Haemophagocytic Lymphohistiocytosis. On examination, the infant had a high-grade fever, light coloured hair, generalized maculopapular rash and hepatosplenomegaly. Routine complete blood count, peripheral blood film and bone marrow examination showed the pathognomonic features of CHS. In view of the grim prognosis and unsatisfactory modality of treatment options, bone marrow transplantation was offered. The infant had an uncomplicated post-transplant period and is well with normal milestones after one year. This presentation is to increase awareness and to highlight the diagnostic laboratory features of this rare condition.

11. **Rapid Detection of Argininosuccinic Acids for the Screening of Argininosuccinase Deficiency**

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Argininosuccinic lyase or argininosuccinase is one of the five enzymes in the Urea Cycle which is responsible for elimination of toxic nitrogenous compounds. It is relatively uncommon in Malaysia compared to other urea cycle defects but its presentation is virtually identical. Biochemical diagnosis is made when elevation of ammonia, glutamine and mildly elevated citrulline are noted in the blood together with elevation of argininosuccinic acids (ASA) in the urine. Previous amino acids detection methods using HPLC with pre-column derivatisation is unable to detect this compound. We have adopted a new amino acids detection method using ion exchange HPLC with derivatisation by ninhydrin where a huge peak of ASA is noted in the patient's urine. However, analysis time is too long (2.5 hours) for one sample. A rapid detection method in the urine is made possible by using electrospray tandem mass spectrometry. 50-ul urine of a known patient is spotted on the S&S filter paper and air-dried. 3 mm punch is made out of that and ASA and other metabolites are extracted using methanol from the urine spot. Derivatization to the corresponding butyl derivatives is done and it is then injected in the tandem mass spectrometry. The butyl ester of ASA (459.3 Da) yields two major fragments at m/z 70 and m/z 144 monitored in the daughter ion scan using narrow mass range (60-150 m/z) which is not present in the normal control urine. Analysis time is only 2 minutes. This approach can be used to screen for ASA in patients with high ammonia.

Poster presentations:

P1. **Enumeration of Absolute Counts of Myeloid and Lymphoid Dendritic Cells in Peripheral Blood using Flow Cytometry**

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Background: Dendritic cells (DC) are the most potent antigen-presenting cells that play a pivotal role in the initiation and regulation of immune responses. Two subsets of DC were identified in peripheral blood known as $CD11c^{+}/CD123^{-}$ myeloid DC and CD $123^{+}/CD11c^{-}$ lymphoid DC. Both subsets differ in phenotype, localization and function. Thus, the ability to identify and enumerate DC is fundamental in understanding the role of these cells in the pathogenesis of human diseases.
Objective: To quantify the absolute counts of myeloid and lymphoid DC in peripheral blood.
Methods: The absolute numbers of myeloid and lymphoid DC were determined by three-colour staining assay using fresh peripheral blood from normal adult volunteers (n = 50). Both subsets of DC were analyzed using FACScan flow cytometer and operated with CellQuest software. Analysis of DC was done within the total white blood cell population.
Results: In the peripheral blood, there was significant difference between the mean absolute numbers of CD $123^{+}/CD11c^{-}$ lymphoid DC and $CD11c^{+}/CD123^{-}$ myeloid DC. $CD123^{+}$ /$CD11c^{-}$ lymphoid DC had a mean concentration of 8.5 ± 2.5 per microlitre but $CD11c^{+}/CD123^{-}$ myeloid DC; 17 ± 5.5 per microlitre.
Conclusion: Simultaneous quantitation of two distinct DC subsets in peripheral blood can be carried out using flow cytometry. Myeloid DC appears to be twice as many than lymphoid DC in circulating blood.

P2. **Comparison of Efficiency of Direct Plating and Size-Sieved Method for the Isolation of Mesenchymal Stem Cell (MSC)**

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Introduction: Adult human bone marrow contains a minority population of mesenchymal stem cells (MSC) that contribute to the regeneration of tissues such as bone, cartilage, muscle, ligament, tendon and fat.
Aim of study: To compare the efficiencies of isolation method between direct plating method and size-sieved method for MSC.
Methods: MSC were isolated from human bone marrow by density gradient fractionation. The isolated mononuclear cells were divided into two aliquots to compare the efficiency of the two techniques for MSC isolation. For direct plating method, mononuclear were directly plated onto a dish supplemented with complete medium. Meanwhile in size-sieved method, cells were plated in a culture device that comprised a plate with 3-mm pores to sieve out MSC from other mononuclear cells. The isolated and expanded cells were characterized at passage 2 to 3 by flow cytometric analysis for specific surface antigens.
Results: MSC isolated from size-sieved method maintained uniform morphology and were significantly greater in number than cells that were harvested after direct seeding marrow cells in dish only.
Conclusion: This study showed that a relatively homogeneous population of MSC could be isolated by a size-sieved method and it can be used to prepare more homogeneous population cells for the study of growth kinetics of the MSC.

P3. **In Vitro Differentiation of Human Bone Marrow Mesenchymal Stem Cells into Neuron-Like Cells**

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Introduction: Human bone marrow-derived mesenchymal stem cells (hMSC) are pluripotent cells, which have been widely studied nowadays due to their ability to differentiate not only into adipocytes, osteocytes and chondrocytes, but also into cells of other lineages.
Objective: To investigate the ability of human mesenchymal stem cells to transdifferentiate into neuron-like cells.
Methods: hMSC were isolated from human bone marrow by using density gradient separation method and then plated in plastic culture flask. The surface antigen expression of hMSC was evaluated by flow cyotmetry. After 3 to 4 passages in culture, hMSCs were induced with neuronal induction medium to differentiate into neuron-like cells. A serum-free medium was used for the induction. After 3 weeks of induction, neuron-like cells were identified by observing the morphology and investigating the expression of nestin by immunocytochemistry.
Results: Flow cytometry showed that hMSC expressed CD29, CD90 and CD105, but did not express CD34, CD45 and HLA-DR. hMSC displayed neuronal morphological characteristics after only 2 weeks in induction medium. Meanwhile, the neuron-like cells demonstrated by immunocytochemistry to positively express nestin, a neuron cell marker.
Conclusion: hMSC can be induced in vitro to transdifferentiate into neuron-like cells.

P4. **In Vitro Expansion and Differentiation of Dendritic Cells from Peripheral Blood $CD34^+$ Haematopoietic Stem Cells (PBSC)**

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Background: Dendritic cells (DC) are specialized antigen presenting cells with unique ability to initiate primary T cell immune responses. It can be differentiated bone marrow (BM), cord blood (CB), monocytes and leukaemic blast cells. In this study, we demonstrated the potential of the Flt-3 ligand in cooperation of SCF to sustain the growth of $CD34^+$ HSC and differentiation of DC from these cells.
Materials & Method: PBSC were obtained from normal donors who had undergone harvesting for allogeneic transplantation with informed consent. These cells were purified by MACS system and cultured with Flt-3 ligand and SCF for about two weeks. The expanded $CD34^+$ HSC were exposed to Flt-3 ligand, SCF, GM-CSF, IL-4 and TNF-alpha to promote DC differentiation. DC derived from expanded HSC were evaluated by inverted microscopy, MGG staining and flow cytometry analysis.
Results: The purified $CD34^+$ HSC prior to culture was found to be $CD34^+$ and $CD45^-$. DC derived from the expanded $CD34^+$ HSC are morphologically indistinguishable from DC generated from other sources. FACS analysis on day-14 DC indicated that approximately 70% were CD1a cells that coexpressed CD86, HLA-DR, CD4 but $CD3^-$, $CD14^-$ and low to negligible level of CD80 and CD83.
Conclusion: Flt-3 ligand and SCF can be used to expand PBSC in vitro and driven to differentiate into immature DC with appropriate cytokines. These immature DC can then be used for further experiments in antigen presenting.

P5. Multidrug resistance (MDR) proteins in acute leukaemia cases in Hospital UKM

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Background: The expression of the multidrug resistance (MDR) proteins is one of the important determinants of outcome of treatment in patients with acute leukaemia. Patients often relapse with unresponsive disease after an initial response to treatment with cytotoxic drugs. Amongst these are the ATP-binding cassette (ABC) superfamily of membrane transporters, including P-glycoprotein (P-gp), various members of the multidrug resistance-associated proteins (MRP), the breast cancer resistance protein (BCRP) and the lung resistance-related protein (LRP). However, the prevalence of MDR protein expression in the acute leukaemia cases in Malaysia is still unknown.
Objectives: The aim of this study was to determine the expression of MDR protein in newly diagnosed acute leukaemias treated at the Hospital Universiti Kebangsaan Malaysia (HUKM). Patients and methods: Blasts were isolated from a total of 34 patients with newly diagnosed acute leukaemias (18 with acute lymphoblastic leukaemia and 16 with acute myeloid leukaemia). The blasts were incubated with the following antibodies: QCRL-3 (MRP1), polyclonal (MRP4), clone 17F9 (P-gp), clone 5D3 (BCRP) and clone-42 (LRP) and analyzed by flowcytometry. Bone marrow samples from non-malignant disorders in adult and children were used as controls.
Results: The analyses showed that 7/34 (21%) patients were positive for P-gp, 9/34 (26%) for MRP1, 25/34 (74%) for MRP4, 25/34 (74%) for LRP and 1/34 (3%) for BCRP. Eighteen out of 34 (53%) patients showed co-expression of more than one MDR proteins.
Conclusion: This study demonstrates that MDR proteins are highly expressed in our acute leukaemia cases. The prevalence of P-gp protein expression (21%) in our cases is comparable with other published reports. However, further studies must be performed to correlate the chemotherapeutic response in our patients with positive MDR protein expression. This is the first study reported locally on the expression of MDR protein in leukaemia patients.

P6. Cloning of Human Erythropoietin (Hepo) Gene and Ligation into Midge® Vector

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Background: hEPO is a glycoprotein hormone that regulates red blood cell production. The administration of recombinant EPO is now widely used for long-term treatment of anaemia associated with chronic renal failure, cancer chemotherapy and human immunodeficiency virus infection. Delivery of this hormone by gene therapy rather than by repeated injections would provide substantial clinical and economic benefits. Human Mesenchymal Stem Cell (hMSC) could be an effective target for gene therapy because they rapidly proliferate in culture and anticipated to be long lived.
Aim of study: The aim of this study involves cloning of hEPO gene and ligation into MIDGE® vector.
Methods: First-strand cDNA was synthesized by reverse transcriptase using the human liver total RNA as template and oligo dT as synthesis primer. The PCR amplification was then carried out using this first-strand cDNA synthesis product as template and specific primers targeted at the 5'-end and 3'-end of the particular gene in a standard PCR reaction. PCR product was then cloned directly into pCR vector and transformed into E.coli. The plasmids from the selected clone were then verified by PCR and restriction enzymes analysis before automated DNA sequencing was carried out. After DNA sequences analysis, the hEPO gene was then subsequently cloned into MIDGE® vector, a minimal-size gene transfer unit for hMSCs transfection.
Results: The construct of hEPO gene had been cloned. The cloned fragment was 597bp and exhibited 100% similarity to those published hEPO gene sequences in GeneBank databases. PCR screening

and restriction enzymes analysis proved that hEPO gene fragment was ligated into MIDGE® vector.
Conclusion: The full-length of hEPO gene had been successfully cloned and ligated into MIDGE® vector, which would subsequently be used for transfection of hMSC.

P7. **Virtual Microscopy for Blood Disorders**

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Introduction: Virtual microscopy is a method of posting microscope images on, and transmitting them over, computer networks. This allows independent viewing of images by large numbers of people in diverse locations. Traditionally, people use a microscope to examine normal and diseased tissues and cells, which are preserved on 'glass slides' and viewed at various levels of magnification. Now, with the advent of computers and broadband Internet connections, microscope slide images can be digitized and posted online. We are able to study magnified images of cells with the click of mouse.
Aim of study: To create a library of virtual slides of blood disorders.
Method: Slides were first examined under microscope with 400X magnification. Contiguous microscopic pictures of glass slides with various blood disorders were captured with Colour View 8 from Soft Imaging System. Captured images were store in respective databases and merged together to form a single composite image by using Analysis Docu software from Soft Imaging System. A report was then created for each slide digitally.
Result: A collection of digital images for 10 blood disorders was established. Each virtual slide comprised 16 high power fields. These fields appear sufficient for a diagnosis to be made of the blood disorder.
Conclusion: In this pilot study, we have established a library of virtual slides of different blood disorders. They may be of use for training as well as quality control exercise.

P8. **Turning of Mesenchymal Stem Cells into Osteoblasts**

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Introduction: Mesenchymal stem cells (MSC) are adult human pluripotent progenitor cells found in bone marrow, cord blood and peripheral blood. They have self-renewal capability without differentiation in long term culture. However, when stimulated under certain conditions, the cells could differentiate into adipocytes, chondrocytes, astrocytes, tenocytes, cardiomyocytes, hepatocytes, neurons, muscle cells, endothelial and endodermal cells. In the current study, we demonstrated a successful in vitro differentiation of mesenchymal stem cells into osteoblasts, which may be used for treatment of bone diseases.
Methods: Mesenchymal stem cells were harvested from the bone marrow of a leukaemic patient and expanded in culture. For osteogenic induction, non-confluent mesenchymal stem cells were incubated in DMEM supplemented with Fetal Bovine Serum, Dexamethasone, Ascorbate and β-glycerophosphate for three weeks. The osteogenic medium was changed twice a week. Calcium mineralization was determined by Alizarin Red S staining, and a gene specific for osteoblasts, which is the osteocalcin gene, was detected by Reverse Transcriptase-Polimerase Chain Reaction (RT-PCR).
Results: After three weeks of incubation in osteogenic medium, numerous crystals were observed to deposit on the mesenchymal stem cells compared to non-induced mesenchymal stem cells. Calcium deposition was stained positively with Alizarin Red S. The result of RT-PCR has seen a band positive for osteocalcin gene.
Conclusions: We have successfully induced the mesenchymal stem cells into osteoblasts. This was confirmed by cytochemical staining and RT-PCR to detect the specific mRNA expressed by the osteoblasts.

P9. **Enteropathogens isolated from Paediatric Patients with Acute Diarrhea**

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Acute diarrhea continues to become a major public health problem in both developing and developed countries, especially among young children. This study was done to determine the enteric pathogens isolated from paediatric patients, age ranging from 3 days to 12 years old, presenting with acute diarrhea. A total of 854 stool samples were collected between October 2002 until November 2003 from Paediatric Unit, Hospital Kuala Lumpur and Hospital Tuanku Ampuan Rahimah, Klang. These stool samples were screened for bacterial and viral pathogens including *Salmonella spp*., *Shigella spp*., *Campylobacter spp*., Rotavirus and Norwalk virus. The conventional culture methods were used for the isolation of bacterial pathogens; XLD and MacConkey plate and Selenite F broth for *Salmonella* and *Shigella* and Campy plate for the isolation of *Campylobacter spp*. while Rotalex (Orion Diagnostica) was used for the detection of Rotavirus. Only 57 of these samples were tested for Norwalk virus using the Enzyme Immunoassay (EIA) method (SRSV-EIA)(Denka-Seiken). From this study, 49 (5.74%) patients were positive for *Salmonella spp*., which include *S.corvallis*, as the most frequent serotype isolated, followed by *S.welterveden*, *S.paratyphi B* and *S.typhimurium*. 10 (1.17%) patients were positive for *Campylobacter spp*., 2 (0.23%) for *Shigella spp*. and 58(6.79%) positive for Rotavirus. 3 (5.26%) out of the 57 samples tested for Norwalk virus were positive using the EIA kit. Findings from this study demonstrate that rotavirus were the most frequently detected enteropathogen among these children.

P10. **Preliminary Report: Serosurvey of Spotted Fever Group Rickettsia, Borrelia Burgdorferi, Ehrlichia Chaffeensis among Wild Rodents trapped in Gunong Stong, Kelantan**

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Serum samples from 32 wild rats captured in Gunong Stong, Kelantan were tested for total immunoglobulins to Spotted Fever Group Rickettsia (TT118), Ehrlichia chaffeensis and Borrelia burgdorferi by the Indirect Fluorescent Antibody (IFA) method. Antibodies towards any one of the antigens were detected in 25 (78%) rodents. Fourteen of the rodents had antibodies that reacted only towards TT118 and 1 towards B. burgdorferi. The rest had antibodies against more than one antigen that is either towards TT118 and B. burgdorferi (9.37%) or TT118 and E. chaffeensis (12.5%) or towards all three antigens (9.37%). Due to the close relatedness of these pathogens, serological cross reactivity is possible. Further tests are being undertaken to confirm the presence of these pathogens in Malaysia.

P11. **Alveolar Rhabdomyosarcoma presenting as Acute Leukaemia**

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Rhabdomyosarcoma (RMS) is the most frequent soft tissue sarcoma in adolescent and young adults, representing 6% to 7% of childhood cancers. Among the frequent site of origin of this highly malignant tumour are head and neck, genitourinary tract and pelvis, and extremities. Histologically, there are two major types of RMS - embryonal (75% of cases) and alveolar. We describe a case of a 5-year old girl who presented with loss of weight, loss of appetite and abdominal pain for one-month duration. Investigations done by the referring centre included a CT scan of thorax to pelvis revealing massive left pleural effusion with multiple lymphadenopathy involving paraeosophegeal, superior mesenteric, celiac and para aortic regions. On arrival at our centre, she was pale, wasted and in respiratory distress. There were left axillary lymphadenopathy with hepatosplenomegaly. There

was decreased breath sound on the left lower zone with dullness to percussion. Peripheral blood film revealed leucoerythroblastic picture with presence of atypical lymphocytes. A bone marrow aspirate showed extensive infiltration by blast-like cells, which were interpreted as acute lymphoblastic leukaemia. Immunophenotyping showed some population of cells (30%) lacking all hemopoietic markers. Excision lymph node biopsy revealed monomorphic population by malignant cells arranged in sheets and lobular pattern. The cells exhibit small, round to ovoid basophilic nuclei with scanty cytoplasm. Immunohistochemical staining showed the malignant cells are strongly positive for desmin, a mesenchymal marker of muscle differentiation and negative for all lymphoma markers. Findings were consistent with alveolar rhabdomyosarcoma. Patient succumbed shortly after initiating chemotherapy following DIVC secondary to sepsis. Approximately 1% of alveolar rhabdomyosarcoma present as a systemic tumor that simulates leukaemia or lymphoma. Thus, metastatic rhabdomyosarcoma needs to be considered in the differential diagnosis in cases of lymphoid infiltration of the bone marrow.

P12. **Donor Chimerism and bcr-abl gene following Stem Cell Transplantation in Chronic Myeloid Leukaemia**

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Introduction: Chronic Myeloid Leukaemia (CML) is a clonal proliferative disorder of haemopoietic stem cells that generally presents in a relatively benign chronic phase and is followed by a terminal blast crisis. A critical step in the pathogenesis involves the translocation between chromosome 9 and 22; and the creation of a new fusion gene called bcr-abl. Stem cell transplantation is currently one of the treatment options.
Objective: To determine the relationship between donor chimerism and the presence of bcr-abl gene transcript in the recipient after allogenic stem cell transplantation.
Materials and Method: A descriptive study was conducted from first June 2002 until 31 December 2003. The study population consisted of all CML patients who had undergone non-myeloablative peripheral blood stem cell transplant at Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur. All patients had their bone marrow aspiration done at diagnosis and day 30, 60 ,100, 130 and 160 post transplant. The samples were analyzed for bcr-abl as well as chimerism status. All the specimens were processed at the Molecular Haematology Laboratory, Hospital Universiti Kebangsaan Malaysia.
Results: A total of nine cases of CML underwent non-myeloablative peripheral blood stem cell transplant in HUKM during the study period. All patients were transplanted during chronic phase. Out of the nine patients, one patient was found to show mixed chimerism at day 30-post transplant and bcr-abl transcript disappearance. The other 8 cases showed that full donor chimerism was correlated with bcr-abl transcript disappearance.
Conclusion: The observational study showed that full chimerism is required for disappearance of bcr-abl transcript. However, it is uncertain why one case showed disapperance of bcr-abl transcript at Day 30 while full chimerism is not achieved.

P13. **Thyroid Hormone Levels in Cord Blood**

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Introduction and Aim: Clinical diagnosis of congenital hypothyroidism (CH) is difficult at birth without neonatal screening. In line with the priorities of the national health services in Malaysia towards preventive medicine, early diagnosis and treatment of CH is emphasised. As per the NACB guidelines, thyroid test results in neonates must be reported with gestation and age-specific reference intervals respectively and each laboratory should establish its own cut off levels according to the method used. In many of the screening programs, early discharge dictate that specimens be drawn before 48 hours. The TSH cut off level used for recall varies between programs. As a general rule,

if the blood is drawn 48 hours after birth, the cut off value for TSH is 10mIU/L whole blood units. For specimens drawn earlier than 48 hours, appropriate cut off values must be established. As neonatal screening in our laboratory is done using cord blood, the aim of this study was to establish the reference range of free thyroxine (fT4) and thyrotrophin (TSH) of cord blood samples in our local population.
Method and Result: Cord blood samples of 409 healthy neonates sent to the laboratory, UMMC, for screening of congenital hypothyroidism were used to determine the reference ranges for thyrotropin and free thyroxine. The measurements were carried out by chemiluminescent immunoassay (Advia Centaur system, Bayer Diagnostics). The non-parametric reference intervals for cord blood free T4 and TSH were 9.6 to 17.0 pmol/L and 2.6 to 15.08mIU/L respectively. These reference ranges for free T4 and TSH were similar to the published values.
Conclusion: For correct interpretation of the levels of thyroid hormone in cord blood it is essential to have reference ranges based on the laboratory's current methods and derived from the local population. In addition, note should be taken regarding the TSH surge that can occur with the birthing process and the cutting of the umbilical cord that partially explains the high TSH levels seen in cord blood.

P14. **Spectrophotometric Method for Screening of Mucopolysaccharidosis in Urine**

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Mucopolysaccharidoses (MPS) develops as a result of deficiency of one of the 11 lysosomal enzymes involved in degradation of glycosaminoglycans (GAGs), resulting in accumulation of GAGs in cells and excess excretion in urine. Measurement of GAGs content in urine is therefore generally used as a screening procedure for MPS. Several assays have been developed in this laboratory in the past. The spot tests are quick and simple but not quantitative. False positive and false negative result might be obtained with this test. Quantitative turbidity test, which is based on interaction of GAGs with cetylpyridinium chloride was later developed and used to replace the spot test. Unfortunately, a lot of false negative results were obtained. We decided to adopt a method based on metachromasia resulting when di-methyl methylene blue (DMB) is used to stain sulfated GAGs. In this method, 30ul of standards and patient samples were diluted with 120ul of deionised water. 825ul of freshly prepared DMB is later added and mixed thoroughly and later read using a spectrophotometer, which is set at 520nm. Standard graph were plotted and used to calculate the value of GAGs in patients' samples. The assay is found to be linear from 0 to 100 mg/L. Within run precision is 8 % and inter run precision is 7%. Estimates range of reference values is less than 60 gm/mol creatinine for age less than a year, and less than 11 gm/mol creatinine for age one to four years. Affected individuals have values ranging from 30 to 70 gm/mol creatinine for 1-4 years of age. False positives results were found in infants less than one year old. The disease is confirmed by high-resolution electrophoresis and enzyme analysis.

P15. **Use of On-Site Drug Testing Devices for Detection of Opiate, Cannabinoids and Amphetamines in Urine**

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The drug abuse problem in the country could be controlled by the implementation of a urine drug detection program. The availability of on-site testing kits with their easy test protocols and instant results will be helpful for the implementing agency which does not have special equipment and trained personnel. We evaluated the usefulness of Acon® one step drugs of abuse test devices for detection of drug in urine by comparing with chromatographic methods currently being used in our laboratory. 200 samples were screen for three main groups of drug currently being abused, namely

opiate, cannabinoids and methamphetamine using the multi drug kit test. All positive samples were confirmed with either thin layer chromatography (TLC) for morphine, TLC for cannabinoids or gas-chromatography mass spectrometer (GC/MS) for Amphetamine Type Stimulant (ATS). Acon® one step drugs of abuse test devices have 100% correlation with GC/MS for ATS, 95% correlation with TLC morphine and 96% correlation with TLC cannabinoids. The results suggest that the kit has the acceptable sensitivity and specificity for drug detection in urine. It is suitable for routine drug screening for centres without facilities or for on-site detection programs.

P16. **Molecular Diagnosis of HB Constant Spring**

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Hb Constant Spring (Hb CS) is a nondeletion alpha thalassemia prevalent in Southeast Asian populations. Hb CS has been seen in the Malays and Malaysian-Chinese. In Malaysia the carrier frequency of Hb CS is 1-4%, thus carrier detection is essential. Hb CS arises from a termination codon mutation (TAA to CAA) in the alpha 2-globin gene. This results in the production of an alpha globin chain with 131 extra amino acids (total 172). The mRNA is long and unstable, thus the output from the alpha-cs gene is greatly reduced. This results in an alpha-thal 2 effect. In our laboratory we detect Hb CS by polymerase chain reaction (PCR). The technique used is an ARMS (Amplication refractory mutation system) technique. We tested 20 samples using the technique. We found this test fast, reliable and most suited for heterozygous carriers.

P17. **Serotype Distribution and Susceptibility Pattern of Streptococcal pneumonia**

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During the year 2002 from March to December, 114 *Streptococcal pneumonia* strains were received from various hospitals for serotyping and determination of penicillin MIC. The strains were isolated from various types of clinical specimens. Typing and/or grouping was done using Pneumotest Plus-Kit obtained from Staten Serum Institute Denmark. The antibiotic susceptibility pattern was determined by modified Kirby-Bauer disk diffusion method and the penicillin MIC was determined by using E-test strip obtained from ABiodisk Sweden. Of the typable strains the most commonly encountered serotype were 19F, 4, 6B and 23F. The majority of strains were susceptible to penicillin. The prevalence of penicillin-insensitive strains appeared to have increased over the year. Of the typable penicillin-insensitive strains majority belonged to serotype 19F. Other serotypes include 1,6A, 6B, 17F and 23F. PFGE analysis of all the serotypes showed that they are unrelated strains.

P18. **Correlation between MRD at Day 28 in Childhood ALL with Clinical and Haematological Characteristics at Diagnosis**

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Introduction: The evaluation of remission using flow cytometry allows better estimate of the minimal residual disease (MRD) after induction chemotherapy in childhood acute lymphoblastic leukaemia. Residual disease in patients with acute leukaemia indicates unfavorable prognosis. Patients in morphological marrow remission (blast cells less than 5%) may have up to 10^{10} leukaemic cells. However by using flow cytometer lower level of residual disease (1 in 10^4 cells) can be detected. It also can be used as a tool to predict relapse if the patient shows a gradual increase in MRD levels on subsequent follows up.

Objective: To study the correlation between clinical and haematological characteristics at diagnosis and MRD status at day 28.
Materials and methods: 38 cases of paediatric patients with B precursor ALL were analyzed. For MRD detection, samples were analyzed with double staining for the possible presence of residual leukaemic cells with the same phenotypic aberrancy at diagnosis.
Results: There was correlation between high level of LDH at diagnosis and MRD positivity at day 28, $p < 0.05$. There was also correlation of marrow findings and MRD positivity at week 12, $p < 0.05$. However, other haematological parameters and clinical findings at diagnosis showed no correlation with MRD status at day 28.
Conclusion: This study showed that high LDH is predictive of MRD by flow cytometry at day 28 for ALL.

P19. **Erythrocyte Sedimentation Rate generated using a Semi-Automated and a Manual System: An Evaluation**

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Objective: This study aims to compare the erythrocyte sedimentation rate (ESR) generated within 30 minutes using the MICROsed System, a semi-automated instrument, and that obtained using the manual Sediplast System within an hour.
Materials and Methods: The ESR of 140 blood samples were analysed simultaneously, using the MICROsed System calibrated at 18°C, and the Sediplast System at room temperature. All analyses were carried out within the same day of blood collection, throughout a period of 14 days.
Results: The coefficients of correlation, calculated by linear regression analysis, were then compared between the MICROsed System and the manual Sediplast method. Excellent correlation was shown between both methods, with coefficient of correlation >0.9 between the results obtained at 18 °C for the semi-automated, and that at room temperature for the manual. Thereafter, readings obtained using the manual Sediplast System were then corrected to 18°C using Manley table of temperature conversion. Having that correction done, similar good coefficient of correlation >0.9 was also obtained between both methods.
Conclusion: The ESR showed very good correlation between the MICROsed System and the manual Sediplast System. We have thus shown that the MICROsed System was able to produce similar ESR results compared to a conventional manual method within a much shorter time.

P20. **Qualitative Chemical Composition of Calculi from Upper Urinary Tract**

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Examination of urinary tract calculi to determine the chemical composition is important, as it would provide information of diagnostic and therapeutic value. Knowing the common chemical composition of renal calculi in a particular country is relevant as it is related to some geographical and environmental factors in addition to other factors. We report the chemical composition of upper urinary tract calculi from 150 patients over a two-year period from Sarawak General Hospital. The patients underwent percutaneous nephrolithotomy and ureteroscopy for the removal of renal calculi and ureteric calculi respectively. The samples received are either in bulk, "staghorn" like or round to oval in shape; or in fragments depending on the surgical procedure the patient underwent and the consistency of the stone. The stones were tested qualitatively on a series of wet chemical 'spot tests' to determine the presence of calcium, magnesium, oxalate, phosphate, carbonate, urate, ammonium and cystine. Calcium oxalate stones are the commonest entity seen in the western countries but less than 20 % of such stones are seen locally. Majority consists of mixed stones with about 60 % containing urate as a constituent whereas relatively pure urate stones are very low. Three percent of

samples show mixtures of calcium oxalate with triple phosphate and another 3 % shows mixture with cystine.

P21. **Extra Pulmonary Tuberculosis - A Case Report**

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Tuberculosis is still a worldwide health problem. The infection in extra-pulmonary tuberculosis is insidious and the symptoms and signs are generally nonspecific. Splenic tuberculosis is a rare entity of extra-pulmonary tuberculosis. We present here a lady who presented with pyrexia of unknown origin and subsequent trephine and splenic biopsies were consistent with tuberculosis. Puan SI, is a 38-year- old Malay lady who was referred from a private medical center for unresolving fever of three months associated with loss of weight, loss of appetite and night sweats. Her husband had recently been treated for pulmonary tuberculosis. Physical examination revealed the presence of hepatosplenomegaly but no evidence of any lymphadenopathy. Respiratory and cardiovascular examination was unremarkable. Chest X ray was normal. Bone marrow aspirate revealed the presence of abnormal lymphoid cells. Subsequently trephine biopsy showed presence of multiple granulomata. HIV status was negative. Imaging studies showed multiple hypodense lesions in the spleen. Biopsy of the splenic lesion was consistent with tuberculosis. She was treated with antituberculous regimen consisting of Ethambutol, Isoniazid, Rifampicin, Pyrazinamide and Pyridoxine and her clinical status has improved. Splenic tuberculosis is rare and may present as hypersplenism or splenic abscess. Sonographically, multiple hypoechoic intrasplenic lesions have been reported. Accurate diagnosis of the aetiology is not always possible on sonography. Ultrasound guided fine needle aspiration biopsy allows a specific diagnosis to be made in most cases. Patients with tuberculous granulomata of the bone marrow present with one or more abnormalities in the peripheral blood. Bone marrow biopsy has the potential to establish the diagnosis in some patients when other tests are negative. Tuberculosis should be kept in mind especially in endemic areas and bone marrow should be examined in case of suspected extrapulmonary tuberculosis infection.

P22. **Acquired Glanzmann Thrombasthemia: A Case Report**

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Glanzmann thrombasthemia is an inherited platelet disorder characterized by lack of platelet aggregation, absence of clot retraction and prolonged bleeding time despite normal platelet count and morphology. Affected patients frequently present with haemorrhages of the skin and mucous membrane. We present here a lady who presented with a few months history of mucocutaneous and musculoskeletal bleeding, and spontaneous bruising. Initial coagulation studies showed result typical of Glanzmann thrombasthemia – prolonged bleeding time, absence of clot retraction and lack of platelet aggregation against ADP. She was treated with intravenous immunoglobulin and oral prednisolone and her condition improved. A repeat coagulation studies a month later showed significant improvement – bleeding time was much improved, clot retraction was good and platelet aggregation also showed much improvement including response to ADP. We were also able to demonstrate the inhibitory effects of the patient’s plasma on aggregation of normal platelets in response to ADP.

P23. **Double Transplantation in a Patient with Multiple Myeloma**

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Multiple myeloma is an incurable plasma cell malignancy that accounts for 10% of all hematologic cancers. For decades the mainstay of therapy has been the use of melphalan and prednisolone; with this regimen, the median survival is approximately 3 years. Recently, important advances were made that have substantially altered the manner in which patients with myeloma were treated. A French randomised trial in previously untreated patients with myeloma showed improved survival with autologous bone marrow transplantation compared with conventional chemotherapy, with 5-year survival rates of 52% and 12%, respectively. At present, the role of tandem (double) transplantation in myeloma is unclear. However, studies have shown improved survival with tandem autologous transplantation. Overall survival with this approach was 68 months. We present here, a patient with multiple myeloma who received double transplantation. Mr MSH was diagnosed to have multiple myeloma in August 2002. He first presented with proptosis and diplopia to the opthalmology clinic in which a MRI was done and showed a retroorbital tumour. A craniotomy and removal of the mass was performed. Histopathologic findings showed plasmacytoma. Bone marrow aspirate and trephine biopsy showed presence of abnormal plasma cells of 36%. Serum and urine protein electrophoresis showed presence of IgG lambda paraprotein and urine Bence Jones proteins. Skull X-ray showed multiple lytic lesions. He was then treated with 6 courses of VAD chemotherapy and followed by autologous and allogeneic peripheral blood stem cell transplantation. At day 60-post allogeneic stem cell transplant, he was in complete bone marrow remission and donor chimerism study using short tandem repeats showed 100% donor chimerism. However, paraprotein remained at low level. He succumbed one month later due to CMV pneumonia.

P24. **Detection of Cytogenetic Aberration with Conventional and Molecular Cytogenetic on Ht-29 Cancer Cell Lines**

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Research pertaining to chromosomes has been ongoing for over a century. Researches perform most cytological analyses on spontaneously dividing cells or one that are cultured to divide in vitro. Today, cytogenetics has significant applied aspects, especially in modern medicine, where it is used to determine whether disease conditions are associated with chromosome abnormalities. However, in many instances, not every chromosomal abnormality can be detected in spite of the available technology. Within the past decade, the emergence of molecular cytogenetics, the combination of cytogenetics and molecular biology technique such as Comparative Genomic Hybridization (CGH) and Fluorescence In Situ Hybridization (FISH), has increased the resolution and application of traditional cytogenetics. CGH is used as a tool to locate possible regions of genetic imbalance in the abnormal cells, which can then be analyzed at high resolution using techniques such as standard FISH. In this study, an HT-29 cancer cell line, which is a monolayer colon adenocarcinoma cell line from ATCC was used for detection of chromosome aberration. The aim of our current work was to identify the karyotypes characteristic of the cell lines and also allow us to establish the technical quality of chromosome preparation from this cell lines. Chromosome analysis of unstimulated short-term cultures was performed on 70-75% confluent HT-29 cell lines according to standard method. Then the finding were confirmed by molecular cytogenetic. On the basis of the results of this study, we concluded that the trisomies usually are present in a large proportion of the cells. Most of the chromosome banding analyses has shown nullicsomic of chromosome 13 and chromosome 8 is generally monosomic. Marker chromosomes were found in most metaphases. These karyotypes were similar to those published.

P25. **Identification of Hemoglobinopathies and Thalassemias by Agarose Gel Electrophoresis**

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Hemoglobin (Hb) is a complex molecule composed of two pairs of polypeptide chains. The Hb spatial structure and other molecular properties depend on the nature and sequence of the amino acids forming the chains. Substitution of amino acids by mutation is responsible for formation of Hb variants, which have different surface charge and electric mobilities, depending on the pH and ionic strength of the buffer. The resulting structural abnormalities are called hemoglobinopathies. Decreased synthesis of one of the Hb chains results in quantitative abnormalities called thalassemias. We report here the identification of some of the common hemoglobinopathies and thalassemias in Malaysia using the Hydrasys system. This system is a semi-automated multi-parameter instrument. The automated steps are sample application, electrophoretic migration, drying, destaining and final drying. The manual steps are handling and preparation of samples, handling the gel, and setting up the instrument. The Hydragel kit is designed for the separation of the normal hemoglobins (A and A_2) and for the detection of Hb variants (S or D, and C or E) by electrophoresis on alkaline agarose gels (pH 8.5). This is used together with the Hydrasys system. The resulting electrophoregrams are evaluated visually for pattern abnormalities. Some of the common hemoglobinopathies and thalassemias identified are: Heterozygous Hb E, Hb S, Hb Constant Spring, and Hb D; Homozygous Hb E and Hb S; Hb H disease; Hb H disease with Constant Spring; Hb E- b thalassemia; Hb Barts hydrops fetalis; b thalassemia trait and major.

P26. **Molecular Detection of Chronic Myeloid Leukemia by Real Time Polymerase Chain Reaction**

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder. Cytogenetic studies show that about 98% of CML patients have the Philadelphia (Ph) chromosome. This chromosomal abnormality results from a reciprocal translocation between chromosome 9, band q34, and chromosome 22, band q11. At the molecular level (as a result of the translocation), the 3' end of the Abelson (*abl*) oncogene is transposed from chromosome 9 to the 5' end of the break point cluster (*bcr*) gene on chromosome 22. This results in the formation of the *bcr-abl* fusion gene that is thought to play a central role in the pathogenesis of CML. We report here a procedure for the detection of the *bcr-abl* gene using real time polymerase chain reaction (PCR). This involves the collection of blood samples using the RNA/ DNA stabilization reagent, mRNA extraction, and t(9;22) detection. The RNA/ DNA stabilization reagent for blood/ bone marrow contains guanidinium isothiocyanate, triton X-100, and a reducing chemical. The reagent causes lysis of blood cells, and inactivation of enzymes like ribonucleases to prevent degradation of RNA. mRNA isolation is done using the magnetic glass particle technology which involves (i) total nucleic acid fraction preparation by their ability to adsorb to glass surfaces, and (ii) mRNA purification by hybridization, capturing by magnetic particles, and magnetic separation. The mRNA is reversed transcribed and the cDNA synthesized is amplified with specific primers to detect the *bcr-abl* gene using the LightCycler t(9;22) quantification kit. The kit is adapted for PCR in glass capillaries using the LightCycler instrument. CML patients with the Ph chromosome were found to be positive for the *bcr-abl* fusion transcripts, while normal patients were negative for the transcript. Hence, real time PCR can be used to diagnose CML.

P27. **Molecular Epidemiology of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in a Malaysian General Hospital.**

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Twenty-four MRSA strains were picked up randomly from Hospital Tuanku Ampuan Rahimah from the duration of May until July 2003 and subjected to DNA fingerprinting by pulsed-field gel electrophoresis (PFGE). The strains were isolated from special care nursery (12 strains), intensive care unit (1), pediatric ward (3), surgical ward (3), hemodialysis unit (1), medical unit (1), orthopedic (2) and accident and emergency unit (1). A total of 6 PFGE types (A, B, C, D, E, & F) were observed. The majority of the strains showed PFGE type A, seen in 18 strains while 2 strains was of PFGE type C. Other PFGE types were represented by a single strain each. PFGE type A was further subtyped into 4 subtypes, which showed the close relatedness of these strains. All strain isolated from the special care nursery were of PFGE type A, which showed that this may have been an outbreak strain infecting the neonates. PFGE type A was also noted in the pediatric ward, which suggests a spread of the outbreak strain from the special care nursery to the pediatric ward via the transfer of patient. The presence of other PFGE types showed that MRSA in this hospital consisted of many other clones but the predominant clone and probably virulent clone is of PFGE type A. DNA fingerprinting of MRSA strains can provide information on strains involved in outbreak and also the endemic strains in each hospital.

P28. **A Rare Haemoglobinopathy in a Malay Family: A Case Report**

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Haemoglobin Lepore is a rare hybrid haemoglobin variant which is generally mistaken for homozygous beta thalassaemia clinically. However the two conditions can be differentiated on careful scrutiny of routine laboratory tests. The aim of this presentation is (i) to increase awareness about this rare haemoglobinopathy among the clinicians and laboratory haematologists and (ii) to show routine laboratory investigations is sufficient to arrive at a presumptive diagnosis. We report a 2- year- old Malay boy who was suspected to have homozygous beta thalassaemia because of severe pallor with hepatosplenomegaly. His complete blood count, peripheral blood film and haemoglobin electrophoresis on cellulose acetate at alkaline pH led us to the presumptive diagnosis of homozygous Haemoglobin Lepore. It is emphasized that a high index of suspicion is needed to arrive at an accurate diagnosis for proper clinical management and genetic counseling.

P29. **Preliminary Report on Mutational Analysis of Cdkn1b Gene in Colorectal Carcinoma**

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CDKN1B gene encodes a nuclear Protein Kinase Inhibitor normally referred to as p27 protein. It is involved in G1 arrest and may also mediate TGF beta-induced G1 arrest. It binds to & inhibits complexes formed by cyclin E-CDK2, cyclin A-CDK2 and cyclin D1-CDK4 and has been shown to exhibit interaction with nucleoprotein NUP50 which is required for degradation of phosphorylated p27Kip1 after nuclear import. Studies have shown there is over expression of p27 protein in mutagenesis and this correlates with the p53 gene mutations in several studies. CDKN1B gene P27 gene is 4.45 kbp long with 3 exons totaling up to 2.58 kbp long. It encodes a protein of 2.2 kDaltons that comprises of 198 amino acids. The aim of this study is to detect the mutations in this gene and evaluate its prognostic value as a tumour marker in colorectal carcinoma. The methodology involves

amplification of the coding regions by PCR and direct sequencing of the PCR products. All sequences with any nucleotide change detected were subjected to forward reverse sequencing for confirmation. Preliminary results have shown single nucleotide polymorphisms (SNPs) in exons 1 but not in exon 2 from tumour tissues. There was a single nucleotide polymorphism at nucleotide 397: T® C in 6 patients, and a T® C/T in 4 patients out of 12 patients analysed.

P30. **Multiplex MIRU-VNTR for the Genotyping of *Mycobacterium Tuberculosis***

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Mycobacterium tuberculosis strains are often genotyped by a method based on the amplification of tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR). In this study, 10 polymorphic loci were amplified by two multiplex PCRs, each using five sets of primers. The primer sets for one of the multiplex assays were obtained from published reports while those for the second multiplex assay were designed using freeware available on the internet and aligned using BLAST in the NCBI website to ensure specificity for *M. tuberculosis*. The optimization of the assays was facilitated with the use of a commercially available Multiplex kit. Detection of PCR products (indicating the number of tandem repeats for each locus examined) was by gel electrophoresis. The multiplex assays were evaluated with reference strains of *M tuberculosis* and Non-Tuberculous Mycobacteria (MTM) as well as wild strains previously typed by monoplex MIRU-VNTR genotyping. The results showed that the multiplex assays were specific for *M tuberculosis,* reproducible, robust and reliable. In conclusion, multiplex amplification increases the convenience and speed of genotyping by MIRU-VNTR for epidemiological studies on tuberculosis.

P31. **Mechanism of Invasion and Intracellular Survival of *B Pseudomallei* in Human Macrophages – An Electron Microscopic Study**

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B.pseudomallei has been shown to persist intracellularly in melioidosis patients until reactivated by a traumatic event or a decrease in immunocompetence. Intracellular replication and localization of the bacterium has been documented in *vivo* as well as *in vitro* in both phagocytic and non-phagocytic cell lines. Mononuclear phagocytes were isolated from heparinized whole blood obtained from a healthy donor using Ficoll Paque (Pharmacia) density gradient centrifugation and subsequently infected with *B.pseudomallei*. Following fixation of cell pellets at varying time points with glutaraldehyde, routine electron microscopy procedure was carried out. We have shown the internalization of *B.pseudomallei* by macrophages via conventional phagocytosis, enclosed within membrane-bound vacuoles or phagosomes. Fusion of lysosomes with phagosomes occurred within 20 minutes of post infection. Ingested bacilli were classified as “intact” or “damaged” on the basis of their ultrastructural features. Our observations indicate that the phagosome-lysosome fusion mechanism in *B.pseudomallei*-infected macrophages failed to ensure complete clearance of the organism. The resistance of some *B.pseudomallei* to lysosomal killing facilitates intramacrophage survival and proliferation, thus giving rise to relapse and recurrence of melioidosis.

P32. **Detection of Human Papillomavirus in Cervical Smears by Consensus and Type-Specific PCR Assays**

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Human papillomavirus (HPV) is believed to be closely associated with the etiology of cervical dysplasia and cervical carcinoma, especially HPV types 16 and 18 which are both considered as high risk genotypes for the development of invasive cancer of the uterine cervix. In this study, an in-house PCR was established for the detection and typing of HPV in cervical smears of patients attending a private practitioner's clinic. A pair of consensus primers, CP-I and CP-IIG flanking the E1 gene was used to amplify a 188 base pair (bp) fragment of HPV 1, 2, 3, 4, 5, 6b, 7, 8, 9, 10a, 11, 12, 14a, 16, 17, 18, 19, 20, 21, 22, 24, 25, 31, 33, 36, 37, 38, 39 and 46. Samples positive in the consensus PCR were then examined for the presence of HPV 16 and 18 with the use of type-specific primers targetting the E6-E7 genes, producing fragments of 499 bp and 172 bp respectively. These in-house PCR assays were compared with a commercially available assay, using the NeoDin HPV Screen/ Type PCR Kit (Neodin, S.Korea). Of a total of 58 samples, 14 were found to be positive with the consensus primers, of which only 8 were confirmed positive in the Neodin assay. Four of the positives were subsequently determined to be type 18 and three type 16 by both in-house and Neodin assays. In conclusion, the consensus and type-specific PCRs are a potentially valuable tool for the rapid detection and genotyping of genital HPV.

P33. **Molecular Typing of Cryptococcus neoformans in Malaysia**

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Cryptococcus neoformans is a yeast-like fungus which, following inhalation from an environmental source, causes respiratory and neurological disease in humans and animals. The organism can cause life-threatening infections in humans, especially in immunocompromised and AIDS patients. The ecological niche is still uncertain for some *C. neoformans* strains. Molecular typing methods are powerful for discriminating microorganism for epidemiology purposes. In this study, polymerase chain reaction (PCR)-based *URA*5 gene restriction fragment length polymorphism (RFLP) analysis method was used for typing of the clinical and environmental strains (isolated from avian's droppings) of *C. neoformans*. The molecular typing showed that of 67 clinical isolates, 48 (71.6 %) were VNI type , 2(3.0 %) were VNII type, 12 (17.9 %) VGI type, and 5 (7.5 %) were VGII type. All the 17 environmental isolates belonged to VNI types. The results shown that VNI type of *C.neoformans* is the predominant molecular type responsible for most cryptococcosis in Malaysia. This study also provides the molecular evidence that avian's droppings could act as a source of infection for cryptococcosis in Malaysia.

P34. **Comparing Apoptosis in Liver Cirrhosis with Hepatocellular Carcinoma by Terminal Deoxynucleotidyl Transferase (TDT)-Mediated DUTP Nick-End Labeling (Tunel) Assay**

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Cirrhosis of the liver, which is frequently associated with and pre-dates hepatocellular carcinoma (HCC), results from continued cell destruction and loss giving rise to compensatory regeneration of the liver cells. It would therefore be interesting to compare the cell loss pattern in cirrhosis and HCC

to determine whether the pattern of cell destruction differs between neoplastic HCC and non-neoplastic cirrhotic liver cells. A preliminary study was conducted at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur to study apoptosis in cases of hepatocellular carcinoma and adjacent benign cirrhotic liver by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay (ApopTag:Serologicals Corporation). 10 cases of HCC with adjacent cirrhosis were histologically reviewed and diagnoses re-confirmed. 4 µm sections were cut from one selected formalin-fixed, paraffin-embedded tissue block of each case and subjected to analysis after pretreatment with proteinase K (20 µg/ml). The number of positive labeled nuclei was visualized using the light microscope and the cases categorized as groups A (positive nuclei in cirrhosis > HCC), B (positive nuclei in cirrhosis = HCC) and C (positive nuclei in cirrhosis < HCC). There were 5 cases in group A, 2 group B and 3 group C, indicating that rate of apoptosis may be higher in cirrhosis compared with HCC. This could support the view that HCC cells have longer lifespans compared with benign liver cells and partly explain for the rapid accumulation of cells in HCC. Nevertheless, these findings are very preliminary and have to be taken in the context that the number of cases is small. In addition, it has to be pointed out that cirrhosis and HCC are morphological entities with many differing underlying aetiological causes. Thus, the pathogenesis of cirrhosis and HCC, and their consequent cell destruction patterns, may still differ with different underlying causes.

P35. Laboratory Diagnosis of Peritonitis - Is the Use of the Bactec Blood Culture System Justified?

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Peritonitis is a common complication of Continuous Ambulatory Peritoneal Dialysis (CAPD). Traditionally, a clinical diagnosis of peritonitis is confirmed by microbiological culture of peritoneal fluid. However, there are many methods of culture with sensitivities ranging from 25% to over 90%. At the Microbiology Diagnostic Laboratory, University Malaya Medical Centre, routine culture procedures detect bacteria or fungi in about 70% of peritoneal fluids from patients with CAPD. In an attempt to increase the rate of positive cultures, the BACTEC 9240 system was used. In this report, the results of 50 peritoneal fluid cultures by the BACTEC 9240 system are compared with those obtained by the routine agar plate culture following centrifugation. The culture positive rates were 86% and 62% respectively, showing the significantly increased sensitivity of the BACTEC 9240 culture method. The higher sensitivity of the BACTEC 9240 cultures is likely to be due to the larger volume of fluid cultured, the longer duration of incubation and addition of antibiotic neutralizing resins into the culture medium. Hence, despite the higher cost, the BACTEC 9240 system is recommended for the routine culture of peritoneal fluid from patients with suspected peritonitis.

P36. Identification of Mycobacterium Species by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis (PRA) of the *rpoB* Gene

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Diseases caused by mycobacterial species other than *M. tuberculosis* (MTB) and *M. Leprae* are becoming increasingly important causes of morbidity and mortality in the world today, particularly in developing and tropical countries. The differentiation between TB and non-TB mycobacterial (NTM) infections allows the early use of appropriate drug treatment. The aim of this study was to set up a PCR-restriction fragment length polymorphism analysis (PRA) using the novel region of the *rpoB* gene to identify the species of Mycobacterium that are not within the MTB complex. A total of 12 mycobacterial reference strains H37Rv, BCG, *M. avium, M. chelonae, M. fortuitum, M.*

intracellulare, M. kansasii, M. gordonae, M. marinum, M. scrofulaceum, M. smegmatis and *M. xenopi* were used to amplify the 360-bp sequence of *rpoB* gene, which is located between the first variable region (VI) and the second conserved region (C2). The amplified DNAs were subsequently digested with restriction enzyme *MspI.* Preliminary results showed distinctive restriction fragment patterns for each of the NTM species but not for those of MTB complex: H37Rv and BCG. *M.fortuitum* and *M.smegmatis* were not distinguishable in conventional gel electrophoresis. Further work is being conducted to resolve this problem with the use of different gel types and concentrations. In conclusion, the PRA procedure is potentially useful for routine application as it does not involve hybridization steps or the use of radioactivity and can be completed within one working day. It should be a reliable and cost-effective method for the differentiation of most mycobacterial species.

P37. **Determination of immunologic memory to Hepatitis B virus (HBV) ELISPOT**

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Systemic vaccination of individuals at risk of exposure to HBV remains the most important measure of controlling the morbidity and mortality associated with hepatitis B infection. In search for a parameter that is predictive of long-term immunity, the relationship between specific antibody production and specific memory B cells in the circulation were analysed. 34 healthy students were recruited to this study and were followed up until they completed their third dose of vaccination. Out of 34 students, only 14 completed the whole series of vaccination. In this study, memory B cells were isolated from the whole PBMC population based on CD27 expression by using a two-step high gradient magnetic cell sorting procedure. Antigen-specific memory B cells were then cultured and assayed by ELISPOT and were compared with serum level of antigen-specific IgG determined by ELISA assay. There is an increment in memory B cell frequencies and antigen-specific IgG in serum after third dose of vaccination, though no direct correlation could be detected between the two parameters. This finding might be useful in giving some insights for the importance of immunologic memory for HbsAg measurement and it's impact on future policy of booster vaccination.

P38. **Clinical relevance of genetic polymorphisms of human CYP2C9 and racial backgrounds in warfarin maintenance dose in Malaysia**

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Cytochrome P450 2C9 (CYP2C9) is the major enzyme involved in the metabolism of S-warfarin. Genetic polymorphisms of CYP2C9 gene have been reported and may play significant roles in pharmacogenomics. The mean daily warfarin maintenance dose for Indians was significantly higher ($p<0.05$) than for the Malays and Chinese. It could be due to genetic polymorphisms of the CYP2C9. We conducted an investigation to determine the CYP2C9 genetic polymorphisms in a portion of the Malaysian population comprising of three major ethnic groups and to investigate whether CYP2C9 allelic variants are associated with warfarin dosage requirement and whether or not they account for the difference in warfarin dosage among three different ethnic groups. Molecular typing of CYP2C9*1, CYP2C9*2 and CYP2C9*3 was carried out in 337 subjects by PCR-RFLP. Case notes were reviewed to obtain patients' clinical data. 298 subjects were identified as homozygous CYP2C9*1 from this study. 11% of subjects were heterozygous for CYP2C9*2 or/and CYP2C9*3 allelic variants. Of the 337 study subjects, only 2 were CYP2C9*3 homozygotes. Our results suggest that CYP2C9*1 is the predominant allele in all the three ethnic groups. Although CYP2C9*2 is very common in Caucasians, they are rarely found in Chinese and Malay ethnic groups. But, it has occurred in Indians with a frequency of 0.0435. The mean daily warfarin maintenance dose for Indians was 6.31 mg. This was significantly ($p<0.01$) higher than 3.25 mg for the Malays and 3.40

mg in Chinese. Among the homozygous CYP2C9*1 genotypes, Indians also required a significantly ($p<0.01$) higher dose of 6.59 mg than Malays (3.24 mg) and Chinese (3.41 mg). A similar finding was obtained in heterozygous CYP2C9*1/*3. The genetic polymorphisms of CYP2C9 gene may contribute to but might not adequately account for all the observed differences in warfarin dosage among Indians compared to Malays and Chinese.

P39. **Investigation of select genomic loci in engraftment/chimerism following allogeneic haematopoietic stem cell transplants (HSCT)**

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Allogeneic transplantation has become a standard therapy in the restoration of a defective lymphohaemopoietic defect in patients with a variety of disorders. In the restorative attempt, post-transplant monitoring based on chimerism analysis is important as a predictive indicator for the engraftment outcome. Chimerism analysis can be done by using suitable genomic markers. Two marker types of interest are the Variable Number of Tandem Repeats (VNTR) and Short Tandem Repeats (STR). This study aimed to determine the set of genomic markers suitable for use in the Malaysian population in monitoring engraftment. Three VNTRs (ApoB, D1S80, and D17S30) and one STR (vWA) were selected. 186 unrelated Malaysians and 21 transplant cases were typed for these loci using PCR and alleles were designed according to known published fragment size. The allelic spectrum was successfully established and the effectiveness of the selected loci as tools for molecular typing for the detection of chimerism was ascertained. Of the four markers investigated, the combined use of three VNTRs were successful for 85% of the transplant cases, whereby the molecular profiles were compared with and found to be in line with the clinical outcome. The vWA marker was found to be unsuitable. Preliminary results from this study point towards a more promising use of VNTRs, both in terms of suitability and cost, in place of more expensive commercial kits.