[Research Targeted to Produce Top 10-20% of Tier 1 ISI/WoS Publication]

UNIVERSITY MALAYA
HIGH IMPACT RESEARCH (HIR)
MOHE GRANT
(CYCLE 3)

APPLICATION FORM

*Please submit a softcopy of the completed form to **hirgrant@gmail.com** AND three (3) hardcopies of this form to the Office of the Vice-Chancellor, Level 9, ChancelloryBuilding, University Malaya, 50603 Kuala Lumpur. **[Incomplete form will be rejected]***

PART I (TO BE FILLED BY PRINCIPAL INVESTIGATOR)

A	**TITLE OF PROJECT:** *Tajuk Projek :* Investigation of the role of the intestinal microflora in the aetiology of inflammatory bowel disease and the impact of Helicobacter pylori status
B	**DETAILS OF PRINCIPAL INVESTIGATOR** *MAKLUMAT KETUA PROJEK*
B (i)	**Name of Principal Investigator:** **Identity card no.:** *Nama Ketua Projek:* *No. Kad Pengenalan :* Professor Lee Way Seah 620128-07-5739
B (ii)	**Position (Please tick (√)):** *Jawatan (Sila tanda (√)):* ■ **Professor** / *Profesor* ☐ **Associate Professor** / *Prof. Madya* ☐ **Others (please specify)** / *Lain-lain(sila nyatakan)* ☐ **Senior Lecturer** / *Pensyarah Kanan* ☐ **Lecturer** / *Pensyarah* --------------------------------
B (iii)	**Faculty /School/Centre/Unit (Please provide full address):** *Fakulti /Jabatan /Pusat/Unit (Sila nyatakan alamat penuh):* Faculty of Medicine / Department of Paediatrics

B (iv)	**Office Telephone No.:** **Handphone No.:** *No. Telefon Pejabat:* 03-7949 2065 *No. Telefon Bimbit:* 016 228 1297 **E-mail Address :** *Alamat e-mel :* leews@ummc.edu.my
B (v)	**Date of first appointment with this University:** *Tarikh mula berkhidmat dengan Universiti ini:* 2 January 1996

B (vi)	**Employment Status (Please tick ($\sqrt{}$)):**
	Jenis Perkhidmatan(Sila tandakan ($\sqrt{}$)):
	
	■ **Permanent** ☐ **Contract (State contract expiry date):**
	    Tetap *Kontrak (Nyatakan tarikh tamat kontrak)*:
	  _____

C	**RESEARCH INFORMATION** / *MAKLUMAT PENYELIDIKAN*

C (i)	**Research Area (Please tick ($\sqrt{}$))**:
	Bidang Penyelidikan (Sila tanda ($\sqrt{}$)):
	
	☐ **A.** **Pure Science**
	                Sains Tulen
	
	☐ **B.** **Applied Science**
	                Sains Gunaan
	
	☐ **C.** **Technology and Engineering**
	                Teknologi dan Kejuruteraan
	
	■ **D.** **Medical Science**
	                Sains Perubatan termasuk Penyelidikan Klinikal (Epidemiology)
	
	☐ **E.** **Natural Sciences and National Heritage**
	                (Geology, Archeology, Taxanomy, Ecology, Biodiversity)

C (ii)	**Duration of this research (Maximum 36 months):**
	Tempoh masa penyelidikan ini (Maksimum 36 bulan):
	
	    From : **_____**
	    Dari :

To : **_____**		
Hingga :		

C (iii)	**Other Researchers: (Must complete LAST COLUMN)**
	Ahli-ahli penyelidik yang lain:

Bil	**Name** *Nama*	**Faculty/ School/ Centre/ Unit** *Fakulti/ P.Pengajian/Pusat/ Unit*	**Academic Qualification/ Designation** *Tahap Kelayakan Akademik/Jawatan*	**Role and Contribution** *Peranan dan Penglibatan*
1	Associate Professor Ida Hilmi	Faculty of Medicine / Department of Medicine	MBBS, MRCP	Co-investigator
2	Professor Goh Khean Lee	Faculty of Medicine / Department of Medicine	MBBS, FRCP	Co-investigator
3	Professor Jamunavani Vadivelu	Faculty of Medicine / Department of Medical Microbiology	PhD	Co-investigator
4	Professor Christopher Boey	Faculty of Medicine / Department of Paediatrics	MBBS, FRCPCH	Co-investigator
5	Dr. Loke Mun Fai	Faculty of Medicine / Department of Medical Microbiology	PhD	Co-investigator
6				

C (iv)	**Research projects that have been completed or ongoing by Principal Investigator for the last three years.** *Penyelidikan yang telah/sedang dijalankan oleh penyelidik utama di dalam tempoh tiga tahun terakhir.*

Title of Research *Tajuk penyelidikan*	Amount & Funding Source *Jumlah & Sumber Kewangan*	Beginning year *Tarikh mula*	Ending year *Tarikh tamat*	No of IS/WoS journal papers produced *Bil jurnal ISI/WoS*
Childhood Rotavirus Gastroenteritis in Malaysia: a 2-centre Study	Merck,Sharp & Dohme Malaysia. RM 125,000	2008	2010	Publication in Tier 1 Journal: • Ling-Sing Ch'ng, Lee WS, Carl D Kirkwood. Detection and character- risation of rare rotavirus G3P[9] strains in children admitted to hospital with severe diarrhoea in Malaysia during 2008 rotavirus season. *Emerging Infectious Disease* 2011;17:948-950. • Another manuscript written • Two more in various stages of preparation
A randomised, double blind, controlled study on the effect of a growing up milk on the occurrence of infections in toddlers (GIANT Study)	Danone Research, the Natherlands RM 300,000	2009	2010	Poster presentation at World Congress of Pediatric Infectious Disease, Melbourne, November 2011. Full paper being written
Analysis of DLG5 Genetic Polymorphisms in Malaysian patients with Crohn disease	UMRG RM 118,000	2010	2011	Chua KH, Lian LH, Kee BP, Thum CM, Lee WS, Ida H, Goh KL. Identification of *DLG5* and *SLC22A5* gene polyporphisms in Malaysian patients with Crohn's disease. ***Journal of Digestive Diseases*** (accepted).

	I certify that all the facts as contained in this application form are true. I agree to abide by the terms and conditions of this HIR/MoHE Research Grant, including the pledge to publish all findings in Tier 1 ISI/Web of Science Journals. **Date :** **Applicant's Signature :** *Tarikh :* *Tandatangan Pemohon :* _____
C (v)	**Summary of Research Proposal** *Ringkasan Cadangan Penyelidikan*

(a) Research background including Hypothesis /Research Questions and Literature Review.
Keterangan latar belakang penyelidikan termasuk kenyataan hipotesis / persoalan penyelidikan dan kajian literatur.

Inflammatory bowel diseases (IBD) are chronic relapsing idiopathic diseases of the gastrointestinal tract (1). The two most common forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), account for significant morbidity and mortality worldwide (1,2). The peak periods for presentation of CD and UC are the second and third decades of life, although both may present at any age (3,4).

Although significant advances in our understanding of IBD have occurred, as yet, the etiology remains unclear. It is currently hypothesized that an initiator, believed to be either gastrointestinal microorganisms or their by-products, in association with a disruption of the gastrointestinal epithelium, stimulates and subsequently drives a dysregulated immune response in genetically predisposed individuals (5). While it is now well accepted that microorganisms play an essential role in the etiology of IBD, despite decades of research, the microorganism or group of microorganisms involved remain elusive. Over the past decade, evidence has accrued to suggest that dysbiosis may have a pivotal role in the pathogenesis of IBD (6). The overall trend appears to be that members from Firmicutes decrease whilst those from Bacteroidetes and Proteobacteria increase in patients with IBD (6). However, although studies have provided significant insights into alterations underlining the intestinal microbiota of patients with IBD, these observations are **not consistent**, and only very general statements—mostly on the phylum level—can currently be made. The major reasons for these inconsistencies relate to poor study design, inappropriate patient and control populations, the site of sampling and the sensitivity and specificity of the techniques used to detect the microorganisms.

Over the last 50 years the incidence of IBD has significantly increased in Western countries, whereas in developing countries the incidence of IBD has been extremely low (2). However, in recent years **the incidence of IBD in Asian nations, including Malaysia, has risen sharply,** an event that has been attributed to rapid modernization and westernization of the population, including the fall in prevalence of *H. pylori* infection (7). In line with this latter view is the finding that in Western and a number of non-Western populations an inverse association has been observed between *H. pylori* infection and IBD development. Indeed in a recent meta-analysis by Luther *et al*, the authors found that 27.1% of IBD patients had evidence of infection with *H. pylori* compared to 40.9% of patients in the control group. The estimated relative risk of *H. pylori* infection in IBD patients was 0.64 (95% confidence interval [CI]: 0.54–0.75) (8). **These results suggest a protective benefit of *H. pylori* infection** against the development of IBD. Currently the mechanism by which *H. pylori* may protect against IBD remains unclear, however, it has been suggested that immune-modulatory properties of *H. pylori* on the innate immune system may play a role (9). Further a study by Luther *et al* have recently shown that ***H. pylori* DNA has the ability to attenuate the severity of chemically induced colitis**, which was linked to the decreased production of Type 1 Interferon production, which is an important pro-inflammatory cytokine (10)

(b) Objective(s) of the Research
Objektif Penyelidikan

Overall Aims

The aim of the proposed studies is to undertake, a well-designed study in which the patient population is carefully chosen to minimize confounding factors, use highly sensitive cutting edge

technology to detect and quantify the intestinal flora in CD and UC patients and controls with the goal of identifying specific bacteria or groups of bacteria that may be associated with CD and UC. In addition we aim to elucidate the protective effect of *H. pylori* on IBD development, by investigating the effect of *H. pylori* infection on the intestinal flora and the immune response in patients (infected/ not infected with *H. pylori)* with CD, UC and controls.

Specific Aims

1. To investigate in Indian, Chinese and Malay children and young adults (aged 2-40 years) newly diagnosed with CD or UC, the composition and activity of their intestinal and fecal microbiota using high throughput sequencing (DNA and cDNA) and compare this with age and ethnicity matched controls, in order to identify specific microorganisms or groups of microorganisms that are associated with the initiation of CD or UC.

2. To determine in the above children and young adults (CD, UC and controls), using seqenom analysis, the presence of specific host genetic polymorphisms that have been reported to be associated with an increased or decreased risk of CD or UC.

3a. To determine theH. pylori status, of the above children and young adults newly diagnosed with CD or UC and controls, using culture and histological examination of gastric biospies. To determine in those shown to beH. pylori positive, the cagA, the EPIYA motif and vacA status of the infecting strain.

3b. To determine ifH. pylori infection, strain type or grade of gastric inflammation impacts on the composition of the intestinal microflora (see aim 4).

4. To study the intestinal and serum immune response within the above children and young adults newly diagnosed with CD or UC and controls, using PCR array analysis. Perform a 4-way comparison between IBD H. pylori positive, IBD H. pylori negative, controlH. pylori positive, control H. pylori negative to elucidate the underlying immune response that may be involved in the protective effect.

(c) Methodology (Provide sufficient technical details for evaluation)
Kaedah penyelidikan

- High throughput sequencing (DNA and cDNA): Determination of the composition and activity of the intestinal and fecal microbiota
- Seqenom analysis: Determination of host genetic polymorphisms
- *H. pylori* status: culture, rapid urease and histology
- *H. pylori virulence factors: PCR and sequencing*
- Gastric pathology: Histological assessment
- Intestinal inflammation: Histological assessment
- *PCDAI measurements*
- Intestinal and serum immune responses:
- Human Common Cytokines RT² *Profiler*™ PCR Arrays and ELISArrays

(d) Flow Chart of Research Activities (Please enclose in the Appendix)

Please see appendix A

(e) Gantt Chart of Research Activities (Please enclose in the Appendix)

Please see appendix B

(f) Milestones and Dates

(g) Expected Results/Benefit
Jangkaan Hasil Penyelidikan

1. *Novel theories/New findings/Knowledge.*

   To identify specific microorganisms or groups of microorganisms that are associated with the initiation of CD or UC

2. *Targeted Output*

	2013	2014	2015	Total
i. **Publication Tier 1**			15	15
ii. **Bright Spark**	0	0	0	0
iii. **Academic Icon**	1	1	1	1
iv. **Patents**	0	0	0	0

   v. List of 5 Tier 1 ISI/Web of Science Journals (Top 10-20% in this discipline)
       1) GUT
       2) American Journal of gastroenterology
       3) Inflammatory Bowel Diseases
       4) Plos ONE
       5) Pediatrics
       6) Journal of Infectious Disease

3. *Specific or Potential Applications.*

4. *Number of PhD and Masters (by research) Students.*

(h) NOMINATION *OF 3 LOCAL NON-UM EXPERTS WHO CAN REVIEW THIS PROPOSAL*
(Please provide Name, Postal Address and Email Address of each)

1. Professor Wong Kum Tong
   Department of Pathology
   University Malaya Medical Centre
   50603 Kuala Lumpur
   Email: wongkt@ummc.edu.my

2. Professor Sanjiv Mahadeva
   Department of Medicine
   University Malaya Medical Centre
   50603 Kuala Lumpur
   Email: sanjiv@ummc.edu.my

3. Professor Yap Sook Fan
   Department of Pre-Clinical Sciences
   Faculty of Medicine and Health Sciences
   University Tunku Abdul Rahman
   Sungai Long Campus
   Jalan Sungai Long,
   Bandar Sungai Long,
   Cheras, 43000 Kajang,
   Selangor, MALAYSIA.
   Tel : +(603) 9019 4722
   Email: yapsf@utar.edu.my

D	**BUDGET** */BELANJAWAN*

	Budget details *Butiran belanjawan*	Amount requested by applicant *Jumlah yang dipohon oleh pemohon*			Justification *Huraian*
		Year 1 *Tahun 1* *(RM)*	Year 2 *Tahun 2* *(RM)*	Year 3 *Tahun 3* *(RM)*	

Please indicate your estimated budget for this research and details of expenditure according to the guidelines attached.
Sila nyatakan anggaran bajet bagi cadangan penyelidikan ini dan berikan butir – butir perbelanjaan lengkap dengan berpandukan kepada garis panduan yang dilampirkan.

	Budget details	Year 1 (RM)	Year 2 (RM)	Year 3 (RM)	Justification
D (i)	**Vote 11000 - Salary and wages** *Gaji dan upah* RA in Malaysia RAs in University of New South Wales, Australia @ A$ 80,000 per year x 3 years = RM 75,000 x 3.2 (exchange rate at March 2012)	RM 29,040 RM 256,000	RM 31,944 RM 256,000	RM 35,138 RM 256,000	**Justify the number of RA's** *Huraikan Bilangan dan Tempoh Pembantu Penyelidik* **Total Vote :** **RM 864,122**
D (ii)	**Vote 21000 - Travelling Expenses and Subsistence/** *Perbelanjaan Perjalanan dan Sara Hidup.* Traveling costs for H Mitchell & N Kaakoush to travel to Malaysia for consultation of research project (inclusive of return airfare and hotel accommodation)	RM 10,000 AU$ 2,500	RM 10,000 AU$ 2,500	RM 10,000 AU$ 2,500	**Provide details for travelling expenses** *Sertakan maklumat mengenai perbelanjaan perjalanan* AU$ 7,500 x 3.2 (exchange rate) = **Total Vote :** **RM 54,000**

	Budget details *Butiran belanjawan*	Amount requested by applicant *Jumlah yang dipohon oleh pemohon*			Justification *Huraian*
		Year 1 *Tahun 1* *(RM)*	Year 2 *Tahun 2* *(RM)*	Year 3 *Tahun 3* *(RM)*	
D (iii)	**Vote 24000 -** **Rental** *Sewaan*	**NIL**	**NIL**	NIL	**Provide Justification** *Sertakan bajet secara keseluruhan* **Total Vote :**
D (iv)	**Vote 27000 -** **Research Materials,** **Supplies & Services** *Bekalan, bahan-bahan dan perkhidmatan penyelidikan* *In Malaysia* Culture Media DNA extraction, PCR sequencing Misc Courier services Histology of slides (60 patients and 60 controls, for both H. Pylori and inestinal biopsies @ RM 20 per slides) *In Australia* Pyrosequencing Sequenome analysis Immune response	 RM 10,000 RM 10,000 RM 5,000 RM 10,000 RM 2,000 AU$ 28,068 AU$ 4,000 AU$ 6,000	 RM 10,000 RM 10,000 RM 5,000 RM 10,000 RM 2,000 AU$ 28,068 AU$ 4,000 AU$ 6,000	 RM 10,000 RM 10,000 RM 5,000 RM 10,000 RM 2,000 AU$ 28,068 AU$ 4,000 AU$ 6,000	**Provide detailed breakdown of Research Materials & Outsourcing Services** *Sertakan pecahan lengkap bagi setiap bahan penyelidikan* Research materials in Malaysia = RM 105,000 Research materials in Australia = AU$ 125,004 = RM 400,012 **Total Vote :** **RM 511,012**

	Shipping of specimens	AU$ 3,600	AU$ 3,600	AU$ 3,600	
	Budget details *Butiran belanjawan*	**Amount requested by applicant** *Jumlah yang dipohon* *oleh pemohon*			**Justification** *Huraian*
		Year 1 *Tahun 1* *(RM)*	**Year 2** *Tahun 2* *(RM)*	**Year 3** *Tahun 3* *(RM)*	
D (v)	**Vote 28000 -** **Maintenance and** **Minor Repair** **Services** *Penyelenggaraan dan* *Pembaikan Kecil yang* *dibeli*	NIL	NIL	NIL	**Provide details of equipment** *Sertakan maklumat mengenai* *peralatan* **Total Vote :**
D (v)	**Vote 35000 -** **Equipment** *Peralatan*				**Provide detailed** **description** **for each item** *Sertakan maklumat bagi* *kegunaan setiap peralatan* **Total Vote :**

		TOTAL AMOUNT *JUMLAH BESAR*	RM 1,429,134

PART II (CONTRIBUTION OVERSEAS COLLABORATOR(S))

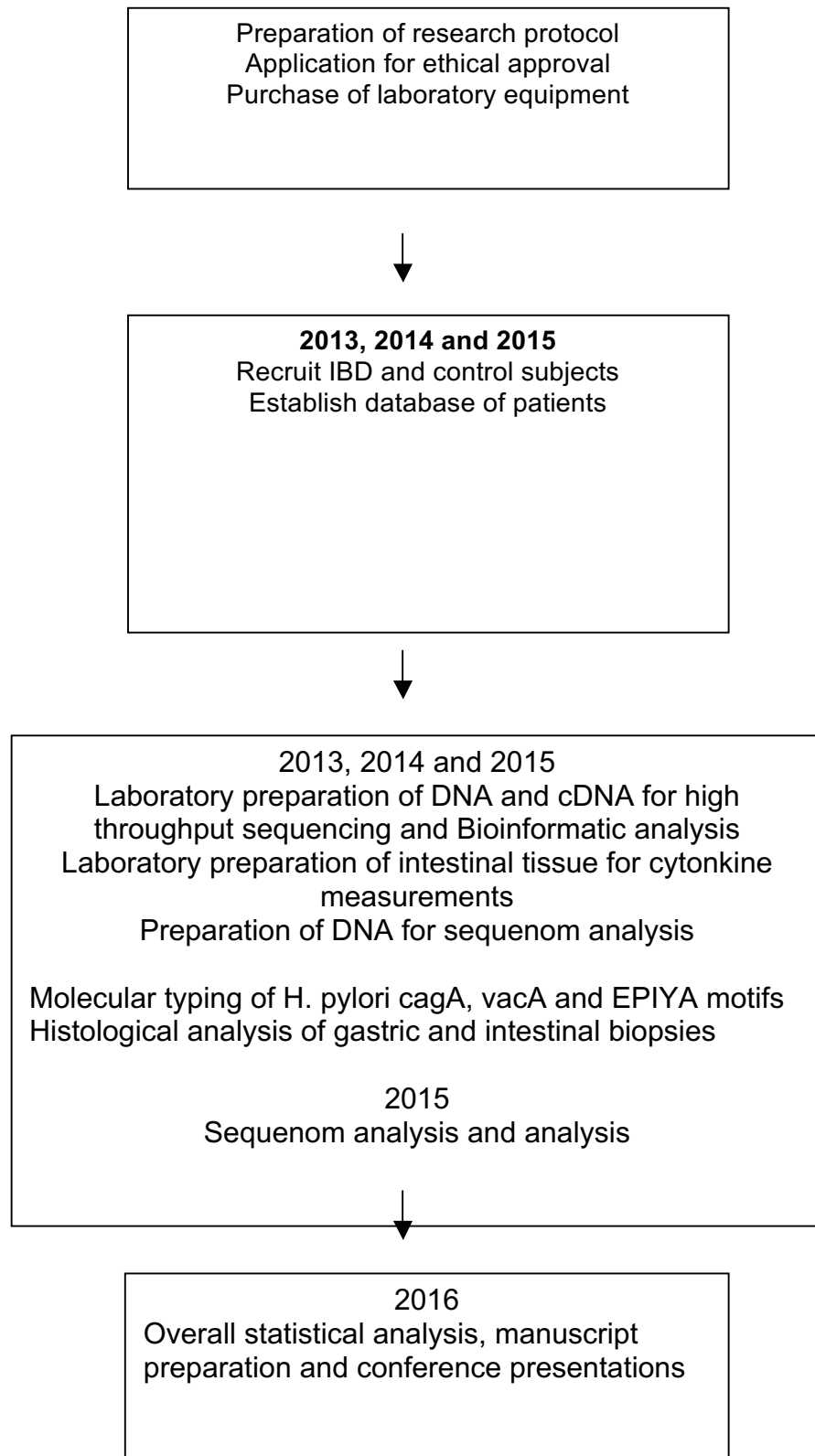| A	**Name of Collaborator :** Professor Hazel Mitchell Dr Nadeem Kaakoush
B	**Address of Collaborator :** School of Biotechnology and Biomolecular Sciences The University of New South Wales Sydney, New South Wales 2052 AUSTRALIA
C	**Contribution by Collaborator :** Suggested study topic Formulated study design Prepartion of the research propsal Co-ordinating studies in Australia Overseeing laboratory aspects of the study in Malaysia and Sydney Supervising and co-supervisng studeny workin on the project Helping to wriite papers
D	**Nature of Technolgy Transfer :** None
E	**Attach Evidence of Agreement to Collaborate :**

Appendix 1: Flow Chart of Research Activities

Preparation of research protocol
Application for ethical approval
Purchase of laboratory equipment

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2013, 2014 and 2015
Recruit IBD and control subjects
Establish database of patients

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2013, 2014 and 2015
Laboratory preparation of DNA and cDNA for high
throughput sequencing and Bioinformatic analysis
Laboratory preparation of intestinal tissue for cytonkine
measurements
Preparation of DNA for sequenom analysis

Molecular typing of H. pylori cagA, vacA and EPIYA motifs
Histological analysis of gastric and intestinal biopsies

2015
Sequenom analysis and analysis

↓

2016
Overall statistical analysis, manuscript
preparation and conference presentations

Appendix 2: GANTT CHART for research activities

ACTIVITIES TOWARDS ESTABLISHING A ONE-STOP CHILD HEALTH CLINIC	2012			2013	2014	2015	2016
	Oct	Nov	Dec	Jan - Dec	Jan - Dec	Jan-Dec	Jan-June
1. Proposal discussed, proforma of studies to be ready, training of research assistants, submission to hospital ethics committee	√	√	√				
2. Recruitment of prospective patients and controls into the trial				√	√	√	
3. Laboratory analysis of tissue samples				√	√	√	
4. Overall Statistical Analysis of data						√	√
5. Preparation of manuscript, and conference presentations							√