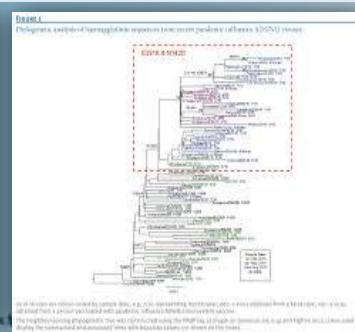
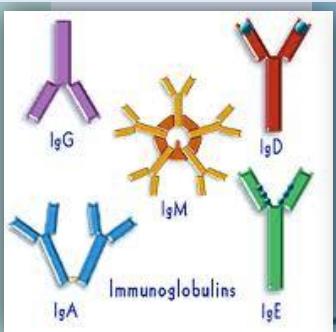
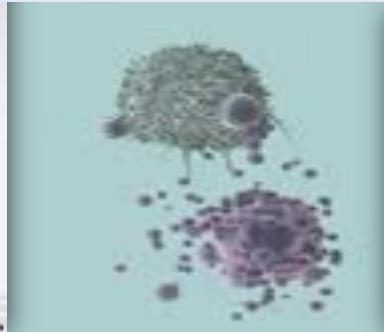
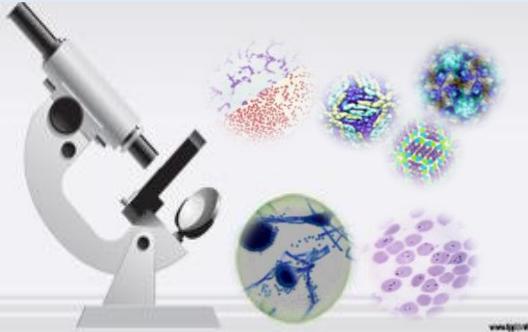


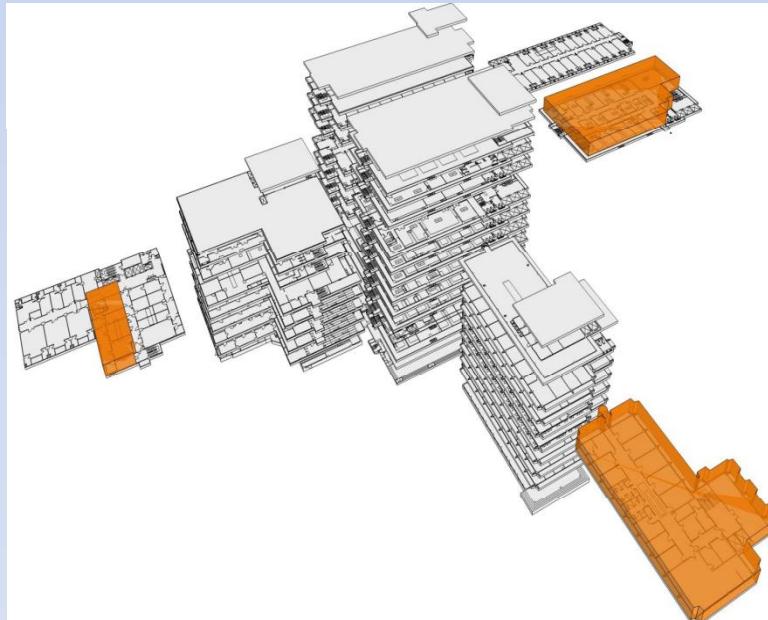
# การนำเสนอผลงาน ภาควิชาจุลชีววิทยาและอุณหภูมโนโลยี คณะเวชศาสตร์เขตร้อน, มหาวิทยาลัยมหิดล

24 ตุลาคม 2557





# ที่ตั้ง



- อาคารจำลองฯ ชั้น 9
  - อาคารเฉลิมพระเกียรติ 50 ปี ชั้น 10
  - อาคารคุณหญิงตรัษฎ์หนักจิต ชั้น 6
- และ 7

ก่อตั้ง เมื่อ 29 มิถุนายน 2509

## หัวหน้าภาควิชา

ศ.ดร.นพ.สวนลักษณ์ ราชวานิช  
รศ.ดร.ประมวล เทพชัยศรี  
ศ.ดร.ศรีสิน ดุสมิทธิ์  
รศ.สร้างด์ ตันติวนิช  
รศ.ดร.มนัส วงศ์สุวรรณ



# บุคลากร

## สายวิชาการ

ศาสตราจารย์	1 ตำแหน่ง
รองศาสตราจารย์	1 ตำแหน่ง
ผู้ช่วยศาสตราจารย์	5 ตำแหน่ง
อาจารย์	5 ตำแหน่ง



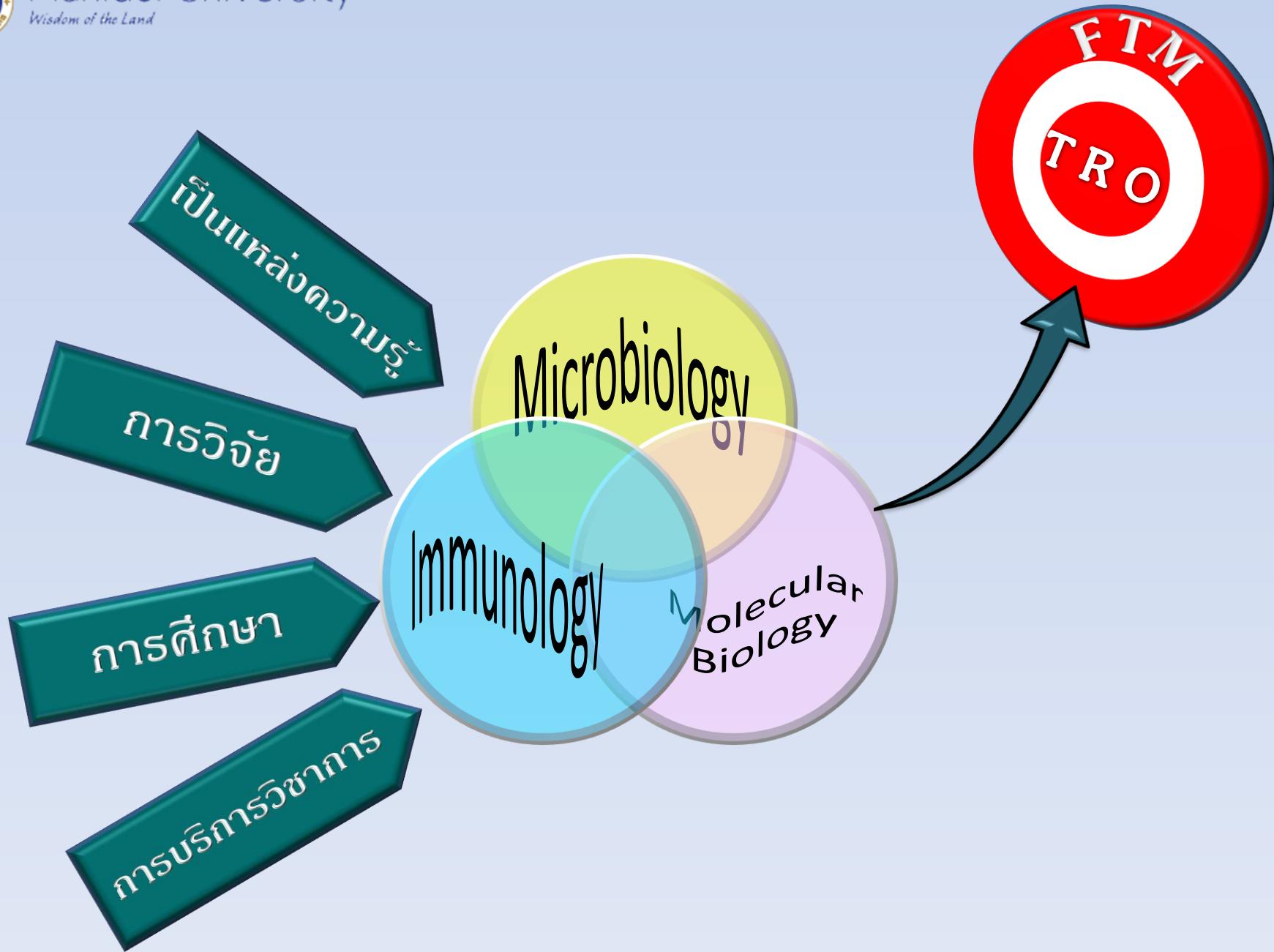
อาจารย์ ลาศึกษาต่อ 1 ตำแหน่ง

## สายสนับสนุนวิชาการ

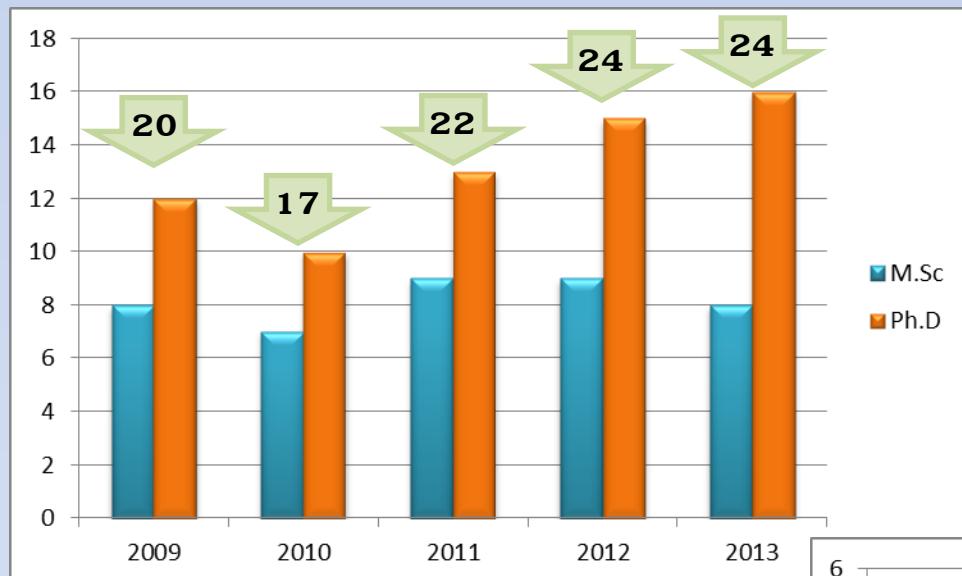
นักวิทยาศาสตร์ (ป.โท)	4 ตำแหน่ง
นักวิทยาศาสตร์ (ป.ตรี)	6 ตำแหน่ง
ผู้ปฏิบัติงานวิทยาศาสตร์ การแพทย์ (อนุปริญญา)	1 ตำแหน่ง

## สายสนับสนุนทั่วไป

เจ้าหน้าที่ธุรการ 2 ตำแหน่ง  
พนักงานสภานัก 4 ตำแหน่ง

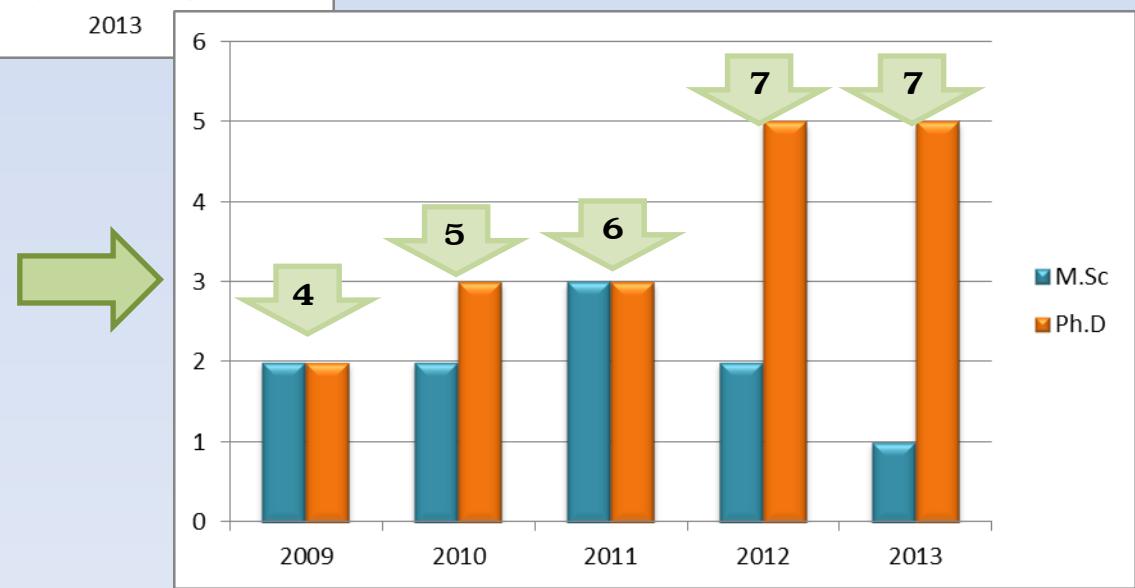


## M.Sc & Ph.D (Trop. Med.)



นักศึกษาสะสม

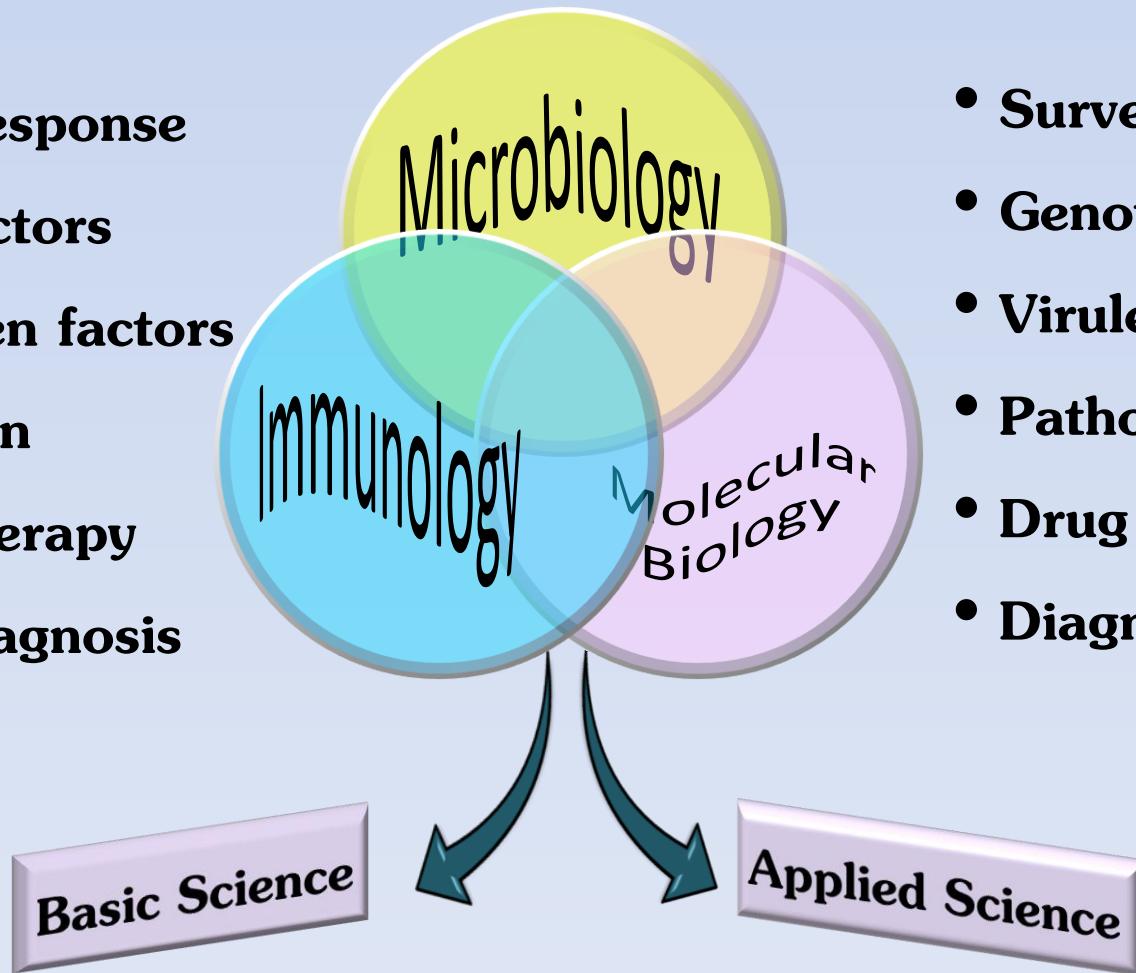
นักศึกษาใหม่





# Fields of studies

- Immune response
- Host factors
- Pathogen factors
- Vaccination
- Immunotherapy
- Immunodiagnosis

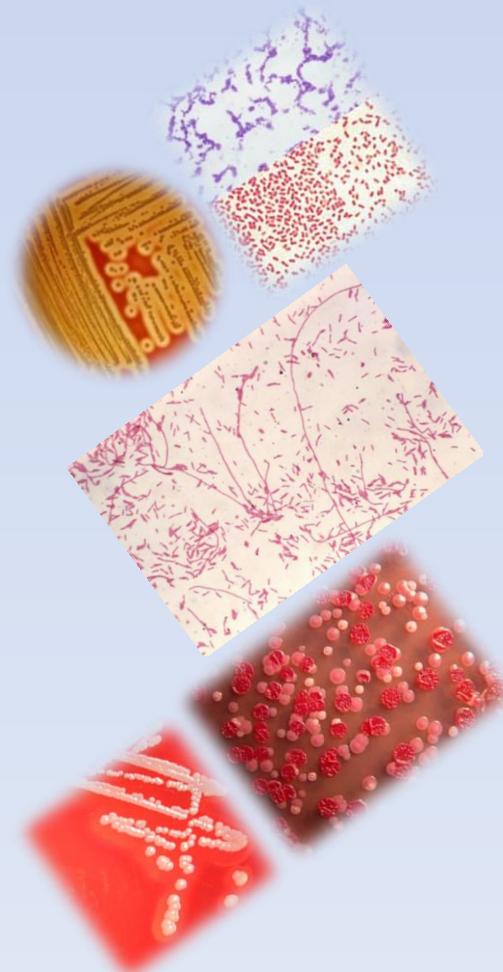


- Surveillance
- Genotyping
- Virulence factors
- Pathogenesis
- Drug resistance
- Diagnosis

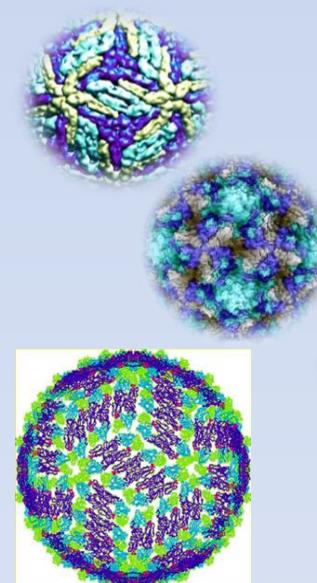


# Microbiology

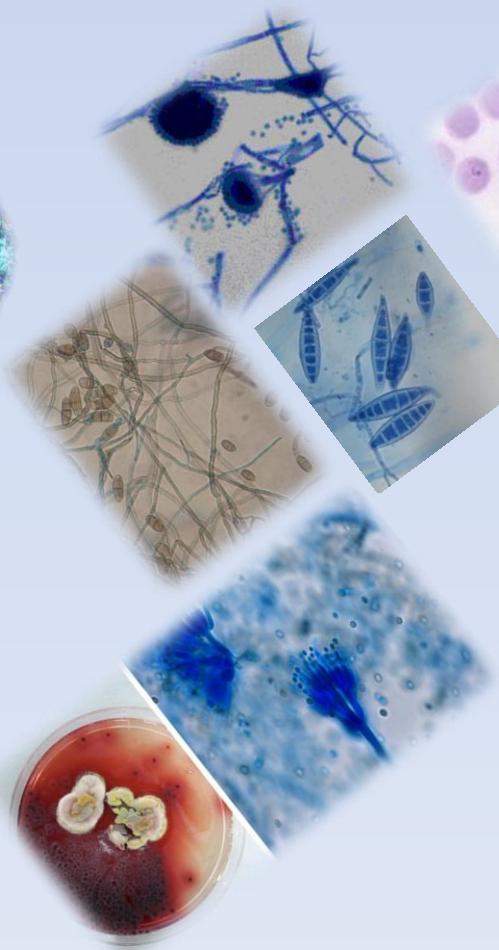
## Bacteria



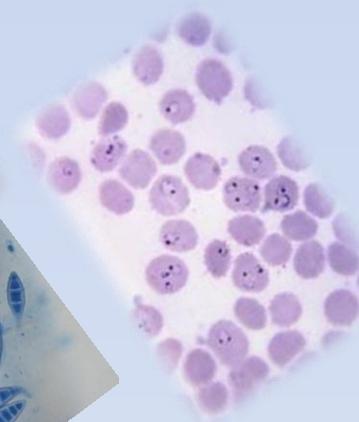
## Viruses



## Fungi



## Malaria



## Others



# Clinical impact of genetic polymorphisms in the innate immune pathway for patients with melioidosis and *S. aureus* infection



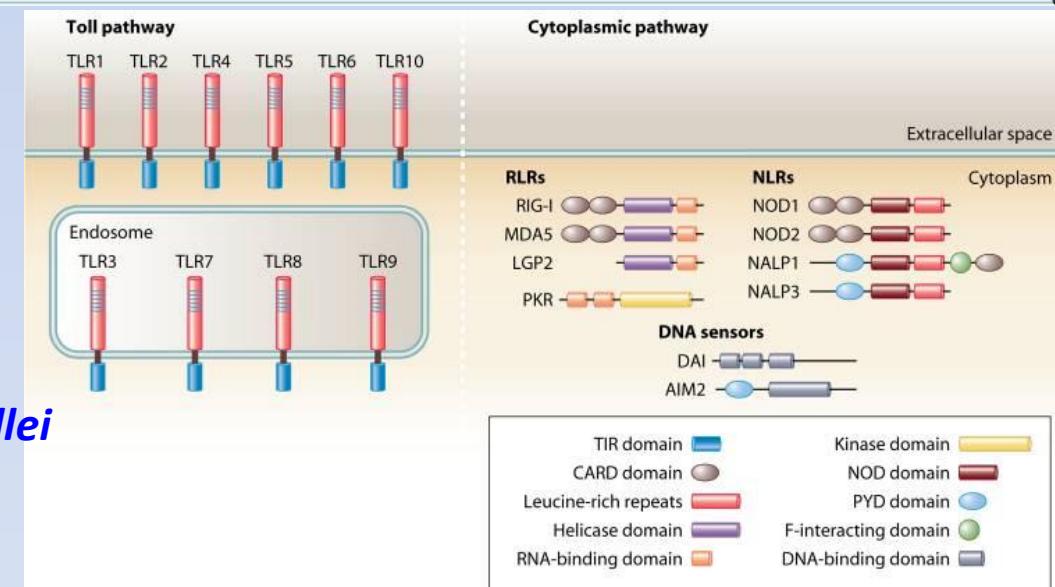
**TLR2 impairs host defense in gram-negative sepsis caused by *B. pseudomallei***

Wiersinga WJ, et al. Plos Med 2007

**TLR4 region genetic variants are associated with susceptibility to melioidosis**

West TE et al. Gene Immune 2012

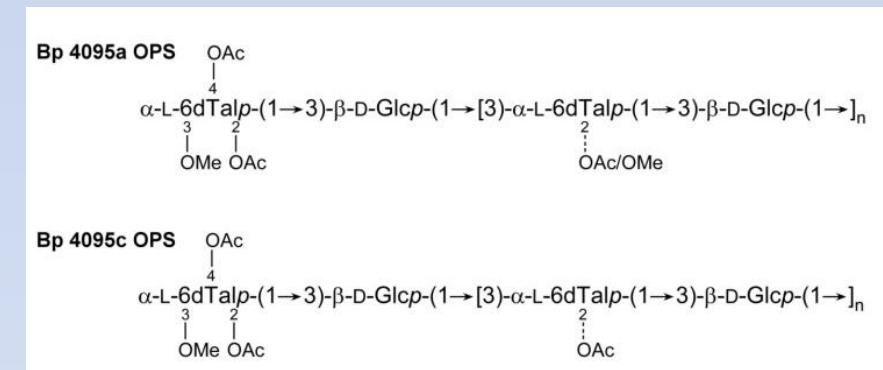
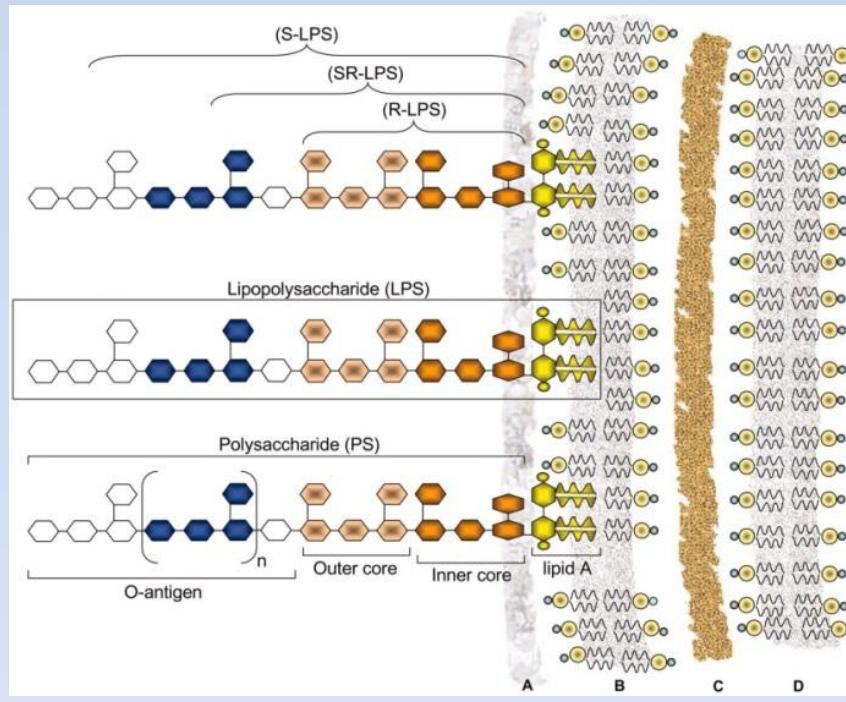
**Screen of whole blood responses to flagellin identifies TLR5 variation associated with outcome in melioidosis**  
Chantratita N, et al. Gene Immun 2013.



**Toll-like receptor 5 functionality is associated with survival in melioidosis**  
West TE et & Chantratita N et al.  
J Immunol 2014

**NOD2 contributes to host susceptibility in murine and human melioidosis**  
Chantratita N, et al. J Immunol 2014

# Investigation of bacterial factors: *B. pseudomallei* LPS stimulates the innate immune response



**Survey of innate immune responses to *Burkholderia pseudomallei* in human blood identifies an important role for lipopolysaccharide.**  
**Chantratita N, et al. Plos One 2013**



# Bacterial virulence factors



## The role of trehalase in stress response and virulence of *Burkholderia pseudomallei*

Trehalose is one of carbon source of *B. pseudomallei*.

The bacteria with the trehalase gene mutation grow defectively and lower ability to survive in mouse macrophage.

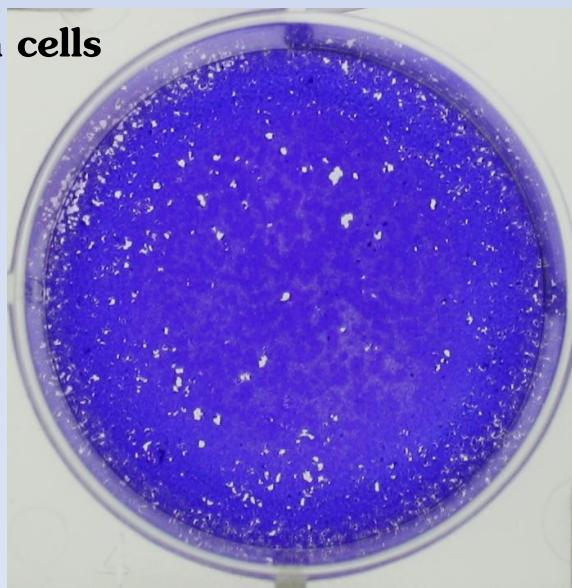


## Pathogenesis mechanism of *Burkholderia pseudomallei*

To study *B. pseudomallei* isolates in aspect of the intracellular survival capability using plaque formation assay



HeLa cells



All *B. pseudomallei* clinical isolates were able to induce severe plaque formation, suggesting the roles of virulence factors in *B. pseudomallei* pathogenesis.

Plaque formation induced by *B. pseudomallei*

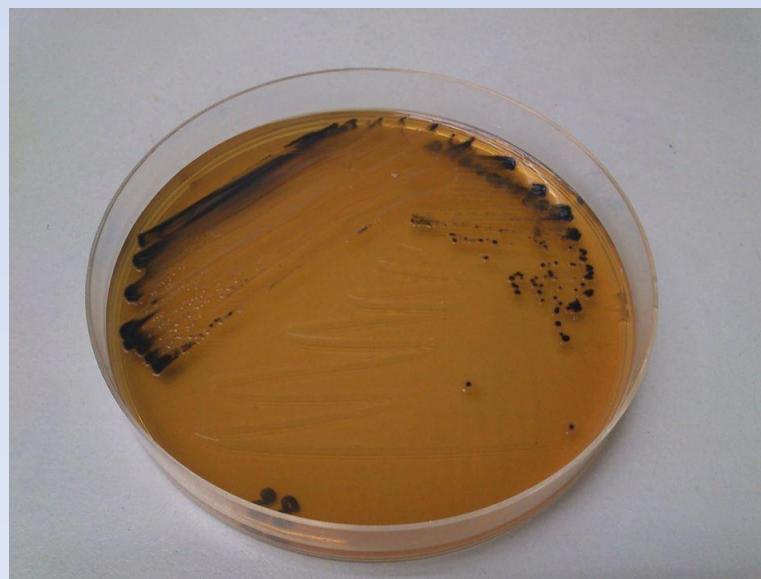


Identify gene associated with pathogenesis.



## Detection of *Salmonella* in the community

To determine the prevalence of *Salmonella* in stool samples of humans, pigs, meat (pork and chicken), and water in the community in Nakhon Pathom Provinces, by conventional culture method



*Salmonella* isolated on SS agar medium

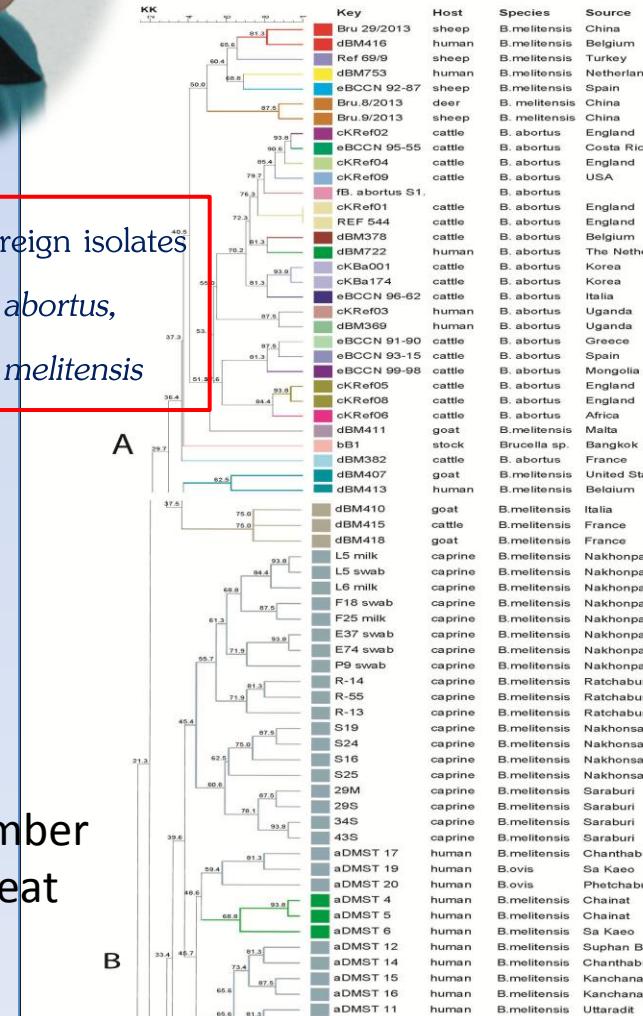
- Less than 1% of pig feces contained *Salmonella* spp.
- Raw chicken (7%) and waste water from pig farms (5%) have been found to carry *Salmonella* spp.

# Bacterial genotyping

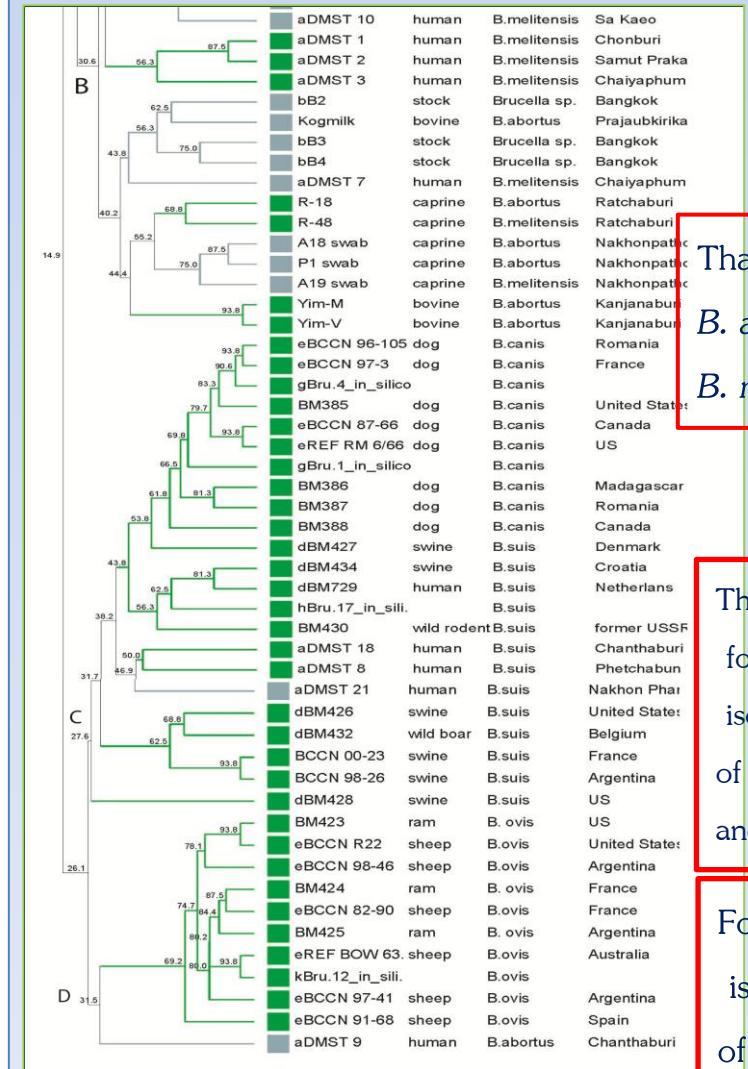


## *Brucella* MLVA-16 genotyping assay

Foreign isolates  
*B. abortus*,  
*B. melitensis*



MLVA =  
multi-locus  
variable-number  
tandem-repeat  
analysis

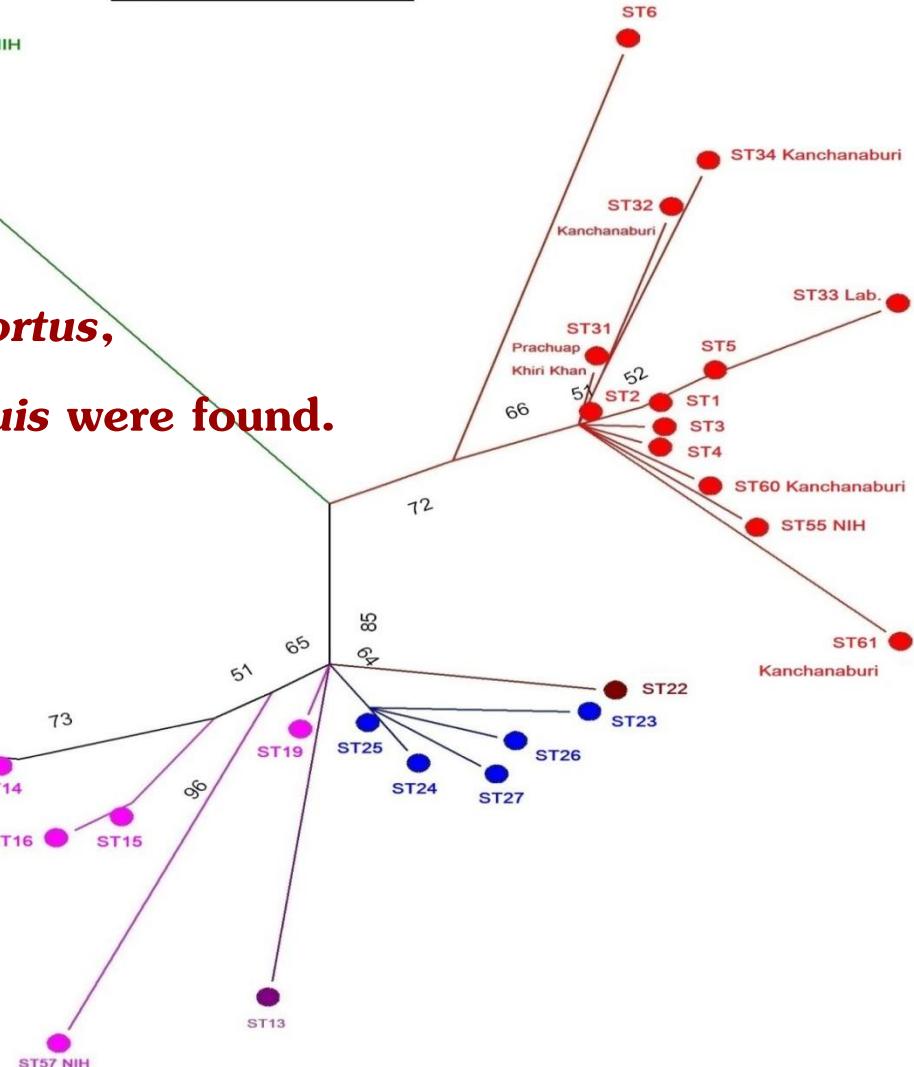
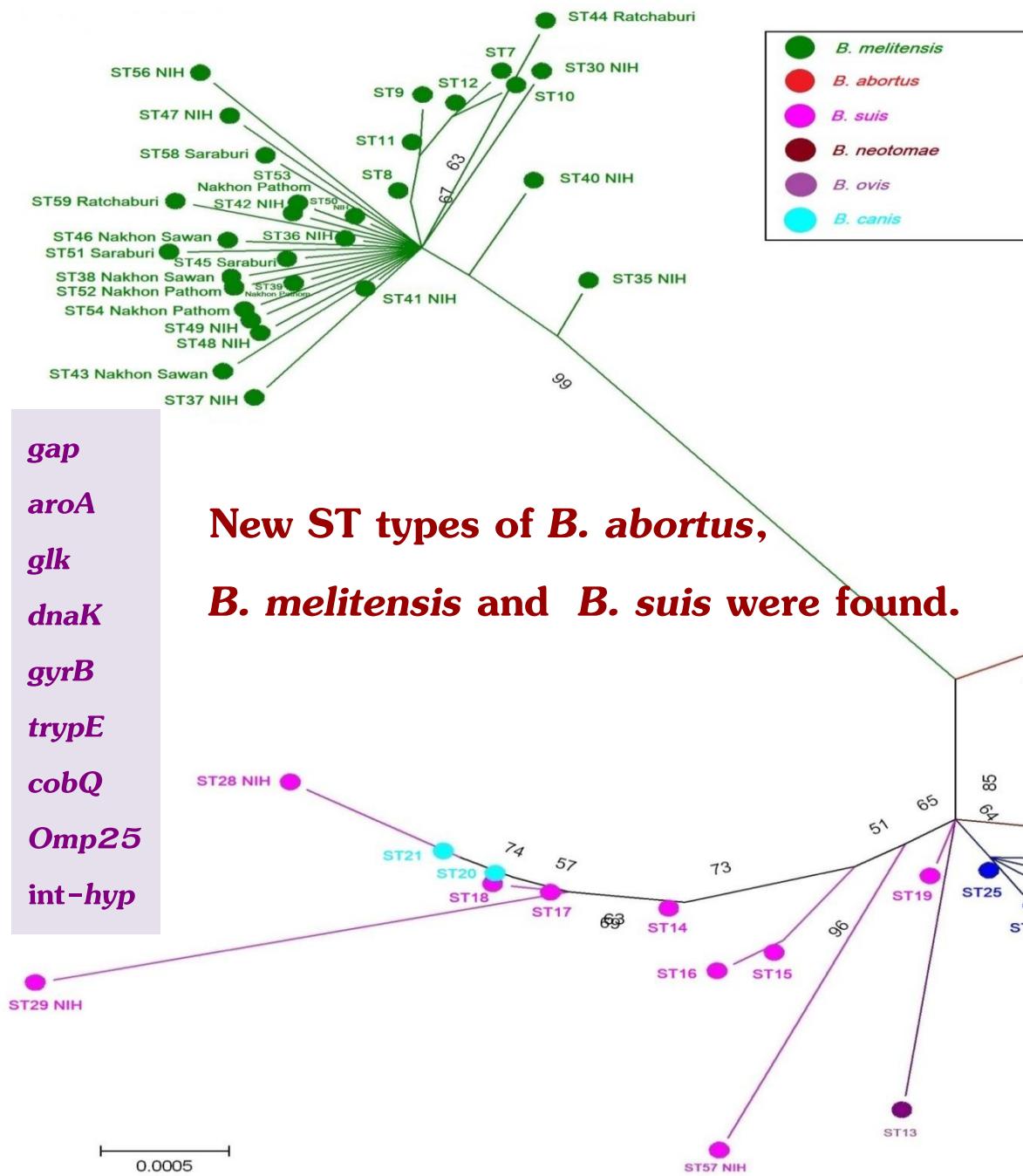


Thai isolates  
*B. abortus*,  
*B. melitensis*

Thai and  
foreign  
isolates  
of *B. canis*  
and *B. suis*

Foreign  
isolates  
of *B. ovis*

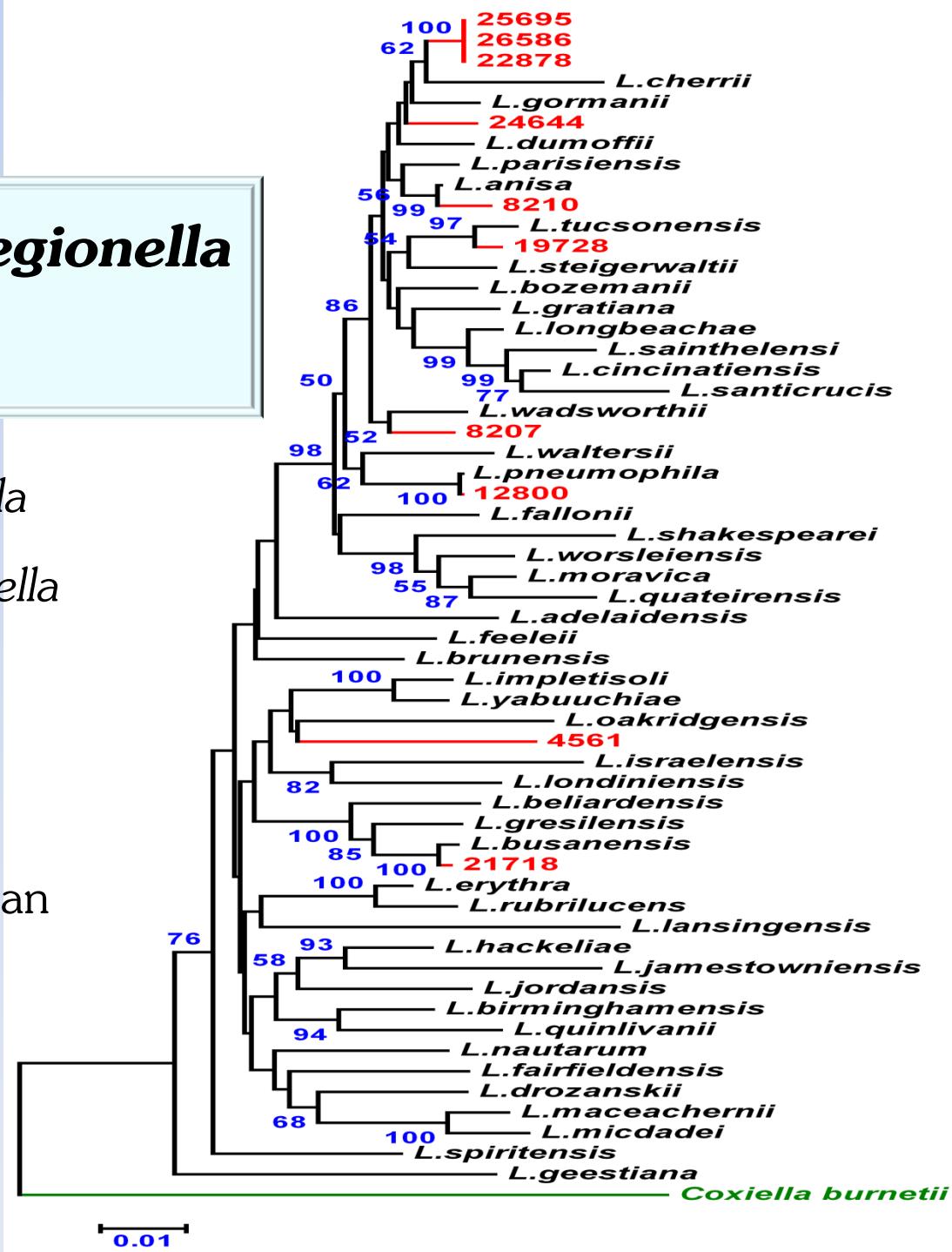
# Brucella genotyping by MLST



# Non-pneumophila *Legionella* genotyping

Phylogenetic tree of *Legionella* isolates and reference *Legionella* species based on 16SrRNA sequences.

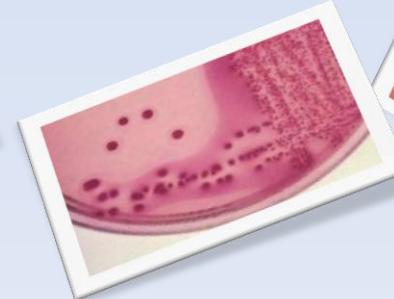
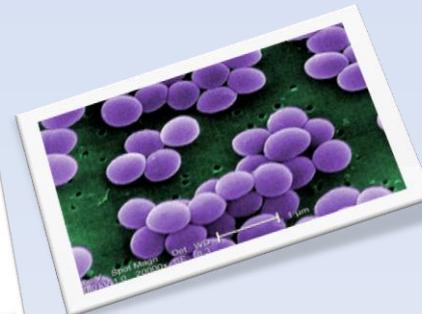
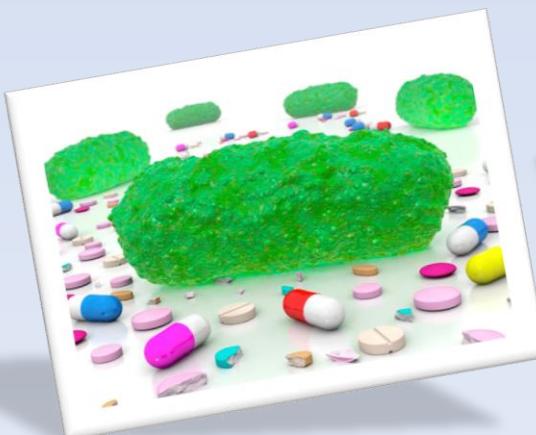
*Coxiella burnetii* was used as an outgroup.



# Drug resistant bacteria

**The antibiotic resistance profile and its mechanism  
in *Escherichia coli* and *Klebsiella pneumoniae* from  
hospital isolations in 2007-2012**

**Epidemiology and genetic analysis of drug resistant  
bacteria in patients, environment and farm animals**





## Immunotherapy

**Preparation of fully human monoclonal antibody to enterotoxin A (SEA) of *Staphylococcus aureus* by using phage display technology for further development to therapeutic antibody**

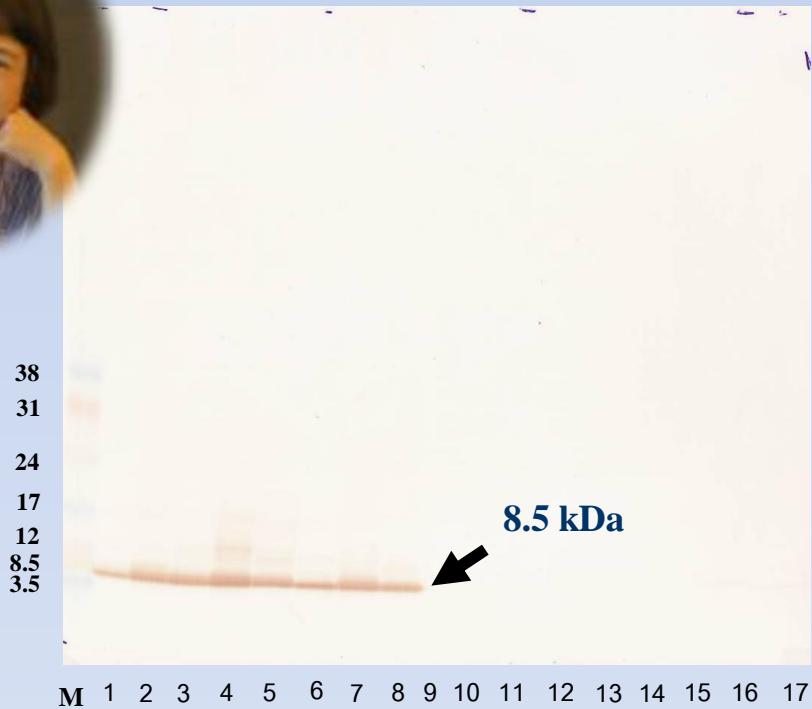
**Preparation of human monoclonal antibody specific to thymic stromal lymphopoietin protein for allergic treatment**

# Immunodiagnosis

## Production of monoclonal antibodies:

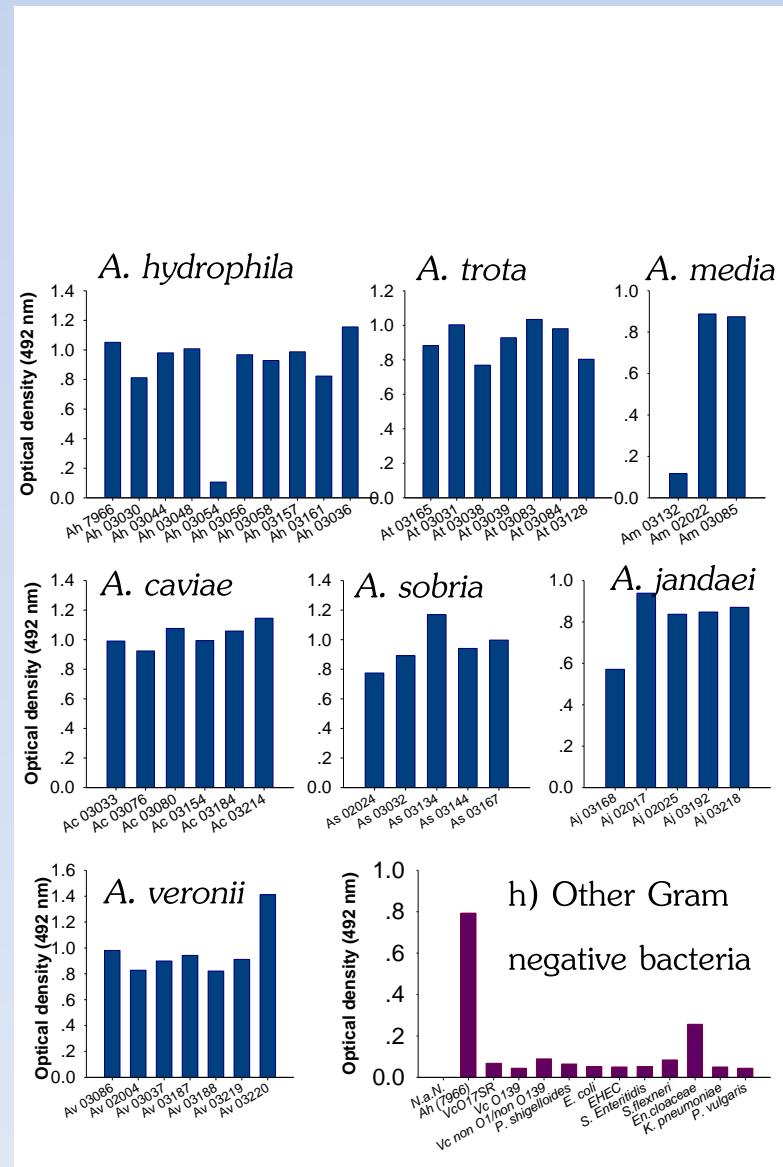
- *Burkholderia pseudomallei* → Latex agglutination  
Immunofluorescent assay
- *Aeromonas* spp. → Dot-blot ELISA
- *Listeria* sp.

# MAb 88F2-3F4 → Aeromonas-specific MAb



**Lane M** = Low molecular weight marker  
**Lane 1** = *A. hydrophila* ATCC 7966  
**Lane 2** = *A. hydrophila* 03036  
**Lane 3** = *A. sobria* 03133  
**Lane 4** = *A. veronii* 03086  
**Lane 5** = *A. caviae* 03125  
**Lane 6** = *A. media* 03132  
**Lane 7** = *A. trota* 03165  
**Lane 8** = *A. jandaei* 03168

**Lane 9** = *Vibrio cholerae* O17SR  
**Lane 10** = *Vibrio cholerae* O139  
**Lane 11** = *Plesiomonas shigelloides*  
**Lane 12** = *Escherichia coli*  
**Lane 13** = *Salmonella Enteritidis*  
**Lane 14** = *Shigella flexneri*  
**Lane 15** = *Enterobacter cloacae*  
**Lane 16** = *Klebsiella pneumoniae*  
**Lane 17** = *Proteus vulgaris*



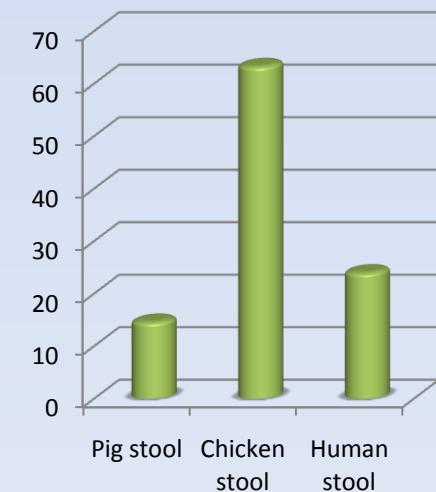


# Viral Surveillance

**Community-based comprehensive, multi-disciplinary surveillance of enteric/food and waterborne pathogens in Kanchanaburi and Nakhon Pathom Provinces, Thailand**

- Objectives:**
1. To determine the most prevalent virus responsible for gastroenteritis
  2. To determine the relationship between the virus strains found in humans and animals.

Type of samples	Total number of samples	No. positive samples					
		Astrovirus	Rotavirus	Enteric Adenovirus	Norovirus GI and GII	Norovirus GII	Total
Pig stool	268	1	0	1	2	35	<b>39 (14.6%)</b>
Chicken stool	30	0	0	0	4	15	<b>19 (63.3%)</b>
Human stool	25	0	0	0	0	6	<b>6 (24.0%)</b>
<b>Total</b>	<b>323</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>6</b>	<b>56</b>	<b>64 (19.8%)</b>





# Viral Surveillance

Molecular Surveillance of Zika virus from Southern part of Thailand

Virus Discovery: Screening of wild caught mosquitoes for flaviviruses  
and blood specimens from febrile patients from Bangkok with  
Dengue-Like Symptoms

Prevalence and diversity of bovine enterovirus (BEV) in wildlife and  
domestic animals in the area of Salakpra Wildlife Sanctuary, Kanchanaburi

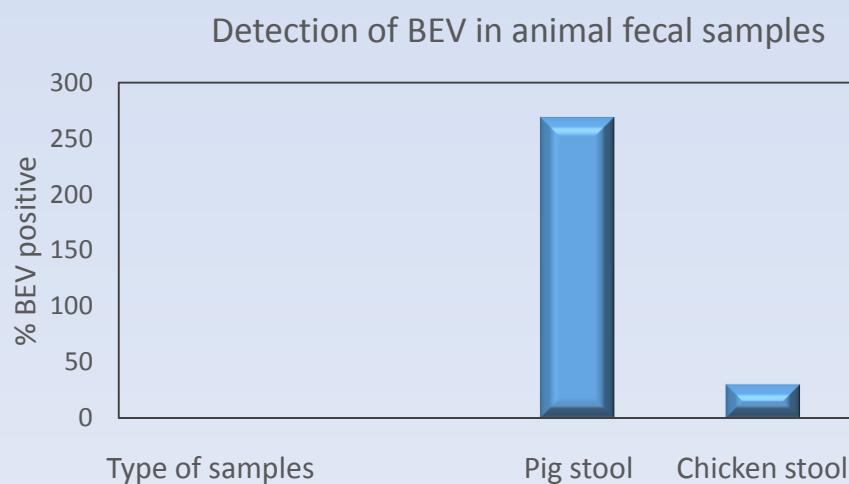




# **Prevalence and diversity of bovine enterovirus (BEV) in wildlife and domestic animals in the area of Salakpra Wildlife Sanctuary, Kanchanaburi**

## **Objectives**

1. To detect the present of BEV genome in fecal samples of wild animals (deer and gaur) and domestic animals (cattle and goat) collected from the area of Salakpra Wildlife Sanctuary, Kanchanaburi, Thailand
2. To phylogenetically analyze diversity and genetic relationship of the BEV in domestic and wild animals





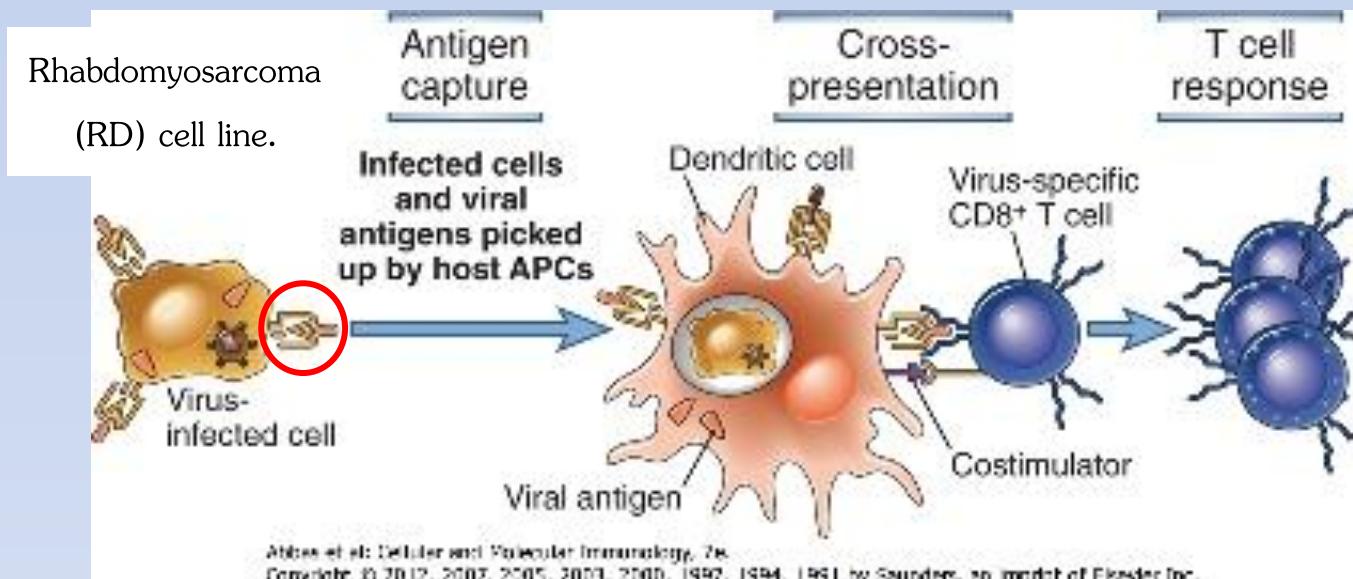
## **Comparison of Reverse Transcriptase PCR and LAMP technique for the detection of Hepatitis E virus in Porcine and Human**

### **Immunoproteomics**

#### **Immunoproteomics for identification of MHC class I-restricted epitopes of enterovirus 71**

#### **Objectives**

1. To develop a mass spectrometry-based immunoproteomics technique for identification of EV71 peptide epitopes presented by MHC class I molecules on infected cells
2. To characterize the immuno-stimulatory function of the MHC class I-restricted EV71 peptide epitopes that are identified by mass spectrometry



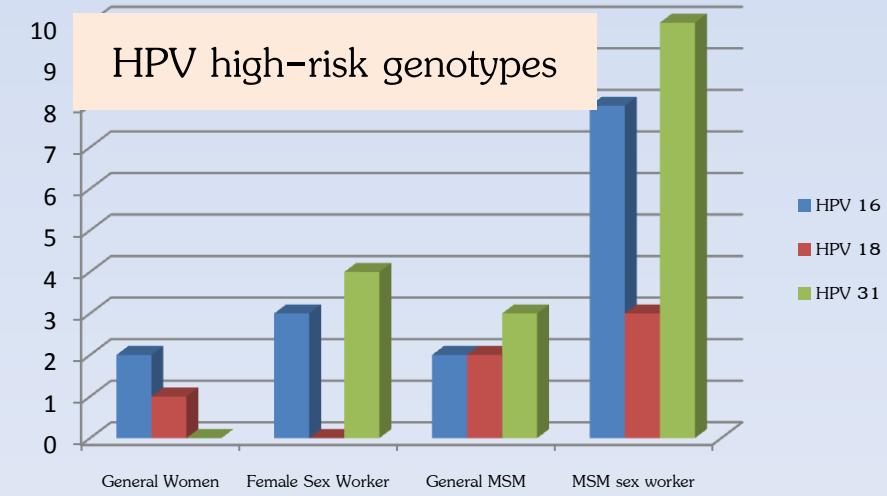
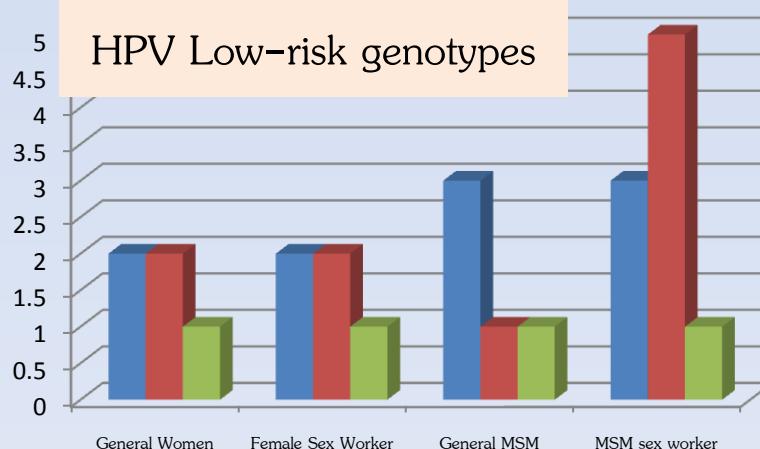
Protein lysate has been prepared from the EV71 -infected rhabdomyosarcoma (RD) cell line.

**Future plan:** MHC class I-bound EV71 epitopes will be separated by immunoprecipitation using pan-MHC class I-specific antibody and subjected for mass-spectrometry analysis.

## Molecular genotyping of HPV L1 gene in low-risk and high-risk populations in Bangkok

### Objective:

To measure the prevalence and genotype distribution of HPV infection among low- and high-risk, male and female groups.



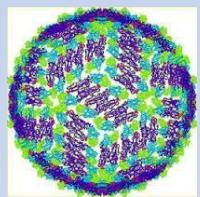


## Viral pathogenesis/therapy

**Dengue-virus-infected dendritic cells trigger  
vascular leakage through metalloproteinase  
overproduction**

### Objective

To demonstrate the molecular basis for DHF/DSS that could be a basis for a general model of haemorrhagic fever-inducing viruses, and identify a new therapeutic approach for the treatment of viral-induced vascular leakage by specifically targeting gelatinolytic metalloproteases.



infect

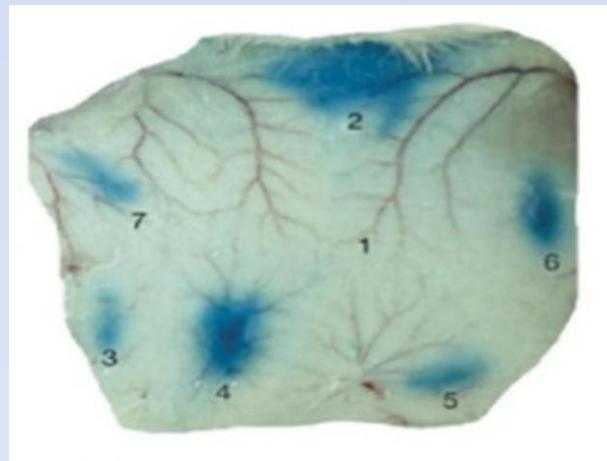
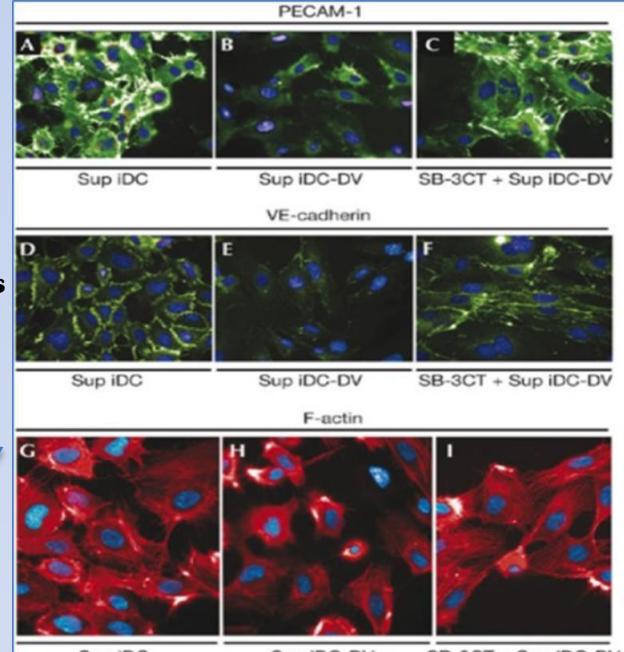


Dendritic cell

Supernatant

HUVECs

MMP



Mouse skin

MMP = matrix metalloproteinase

HUVECs = human umbilical vascular endothelial cells



# Tuberculosis and TB/HIV Coinfection

**Host immune response and gene studies in  
TB and TB-HIV coinfection**

**The immunological and molecular biological aspects  
of tuberculosis and HIV/TB co-infections**



# Research in Pathogenic Fungi

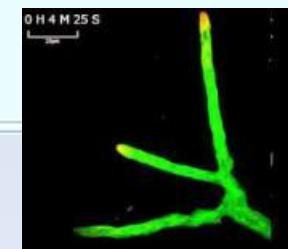
## Virulence factors

The correlation between N-myristoyl transferase (NMT) expression and hyphal growth

Study the interaction of pyocyanin, quorum sensing molecule from *Pseudomonas aeruginosa*, to variation phenotypic of dimorphic fungi, *Penicilium marneffei* and *Histoplasma capsulatum*

Spitzenkorper phenomenon in fungal invasion property of *Candida albican*

<http://www.youtube.com/watch?v=ZIutFBqI6GM>





## Drug resistant fungi

Comparative proteomic analysis of differentially expressed proteins between azole-resistant and azole-susceptible *A. fumigatus*-biofilm.

Role of ER stress in *Aspergillus* drug resistance and their pathogenesis



## **Human Malaria : *P. falciparum*/*P.vivax***

**Host immune response and gene studies in malaria**

**Toll like receptors and innate immunity in malaria**

**Role of innate immune cells Th1 and Th2 cytokine and cytokine receptor gene polymorphisms in relation to functional changes in severe and mild malaria**



## Vaccine development

Duffy-binding protein II among Thai *P. vivax* (PvDBPII)

Phylogenetic analysis of Thai *P. vivax* isolates to others found internationally demonstrated six distinct allele groups.

**Allele groups 4 and 6 were unique to Thailand**



# Mouse Malaria : *P. yoelii* (Py)

Antibody to MSP1<sub>19</sub> induce malaria protection in mice



Py pRBC

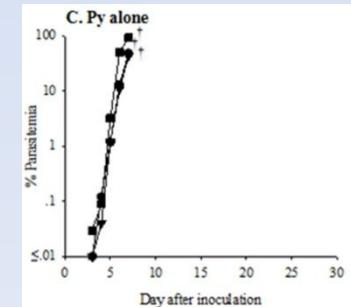
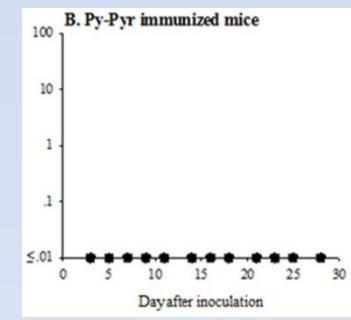
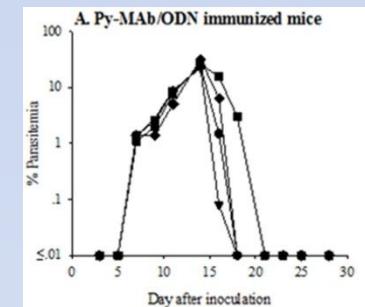
nRBC

A) Treat with MSP1<sub>19</sub> specific antibody and CpG ODN

B) Treat with pyrimethamine

C) No Treatment (control)

D) Treat with MSP1<sub>19</sub> specific antibody and CpG ODN





# Mouse Malaria : *P. yoelii* (Py)

**Antibody to MSP1<sub>19</sub> induce malaria protection in mice**



Py pRBC

nRBC

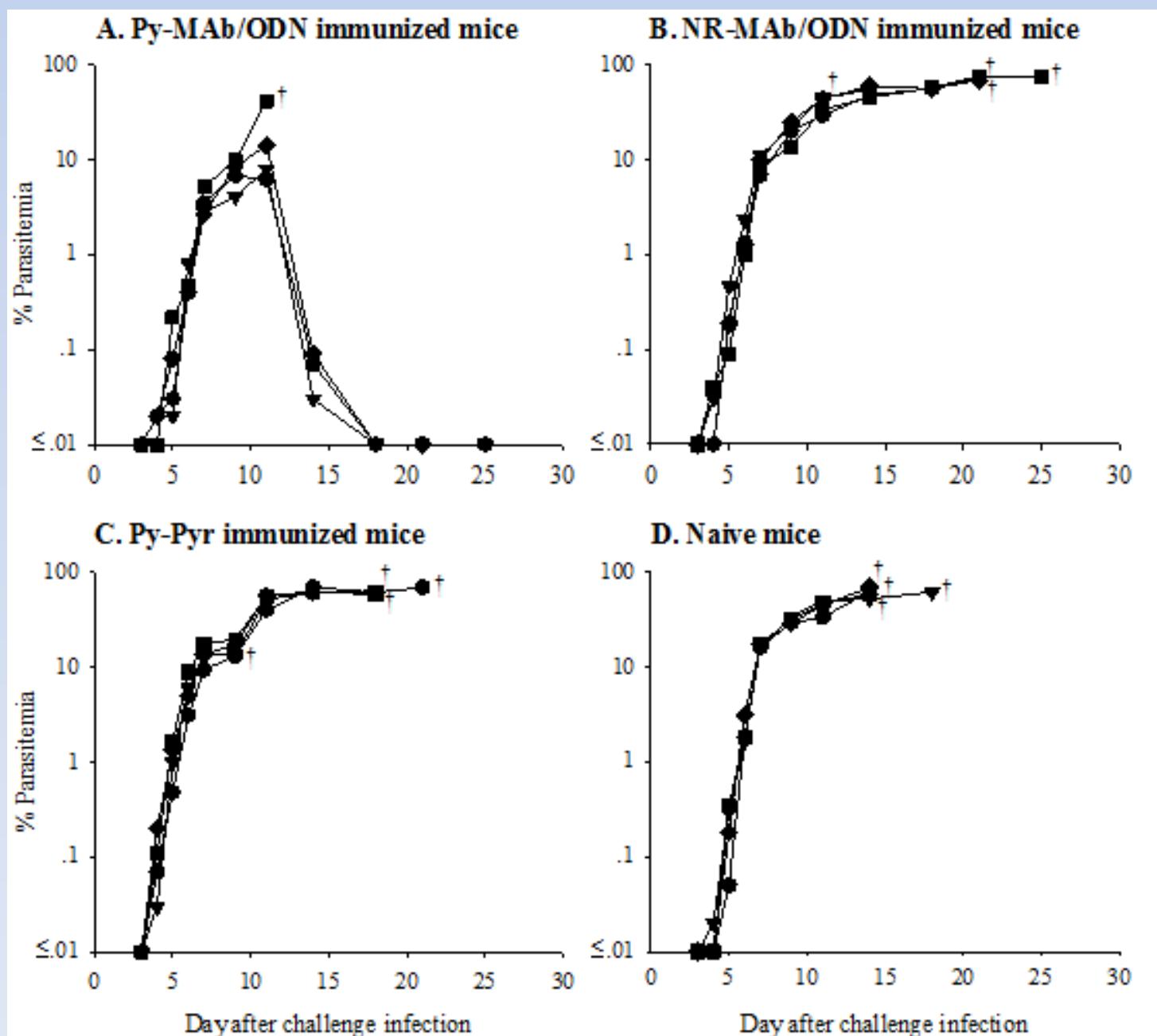
Treat with MSP1<sub>19</sub> specific antibody and CpG ODN

Treat with pyrimethamine

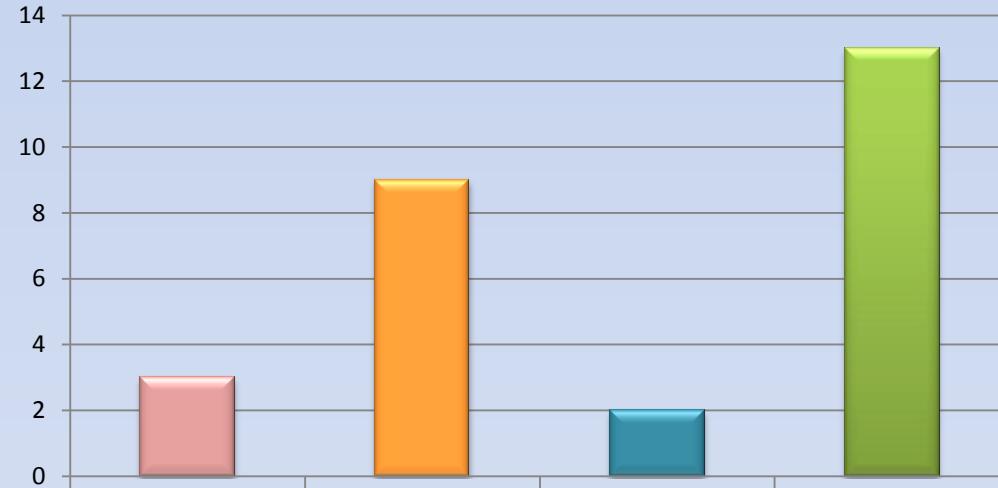
No Treatment (control)

Treat with MSP1<sub>19</sub> specific antibody and CpG ODN

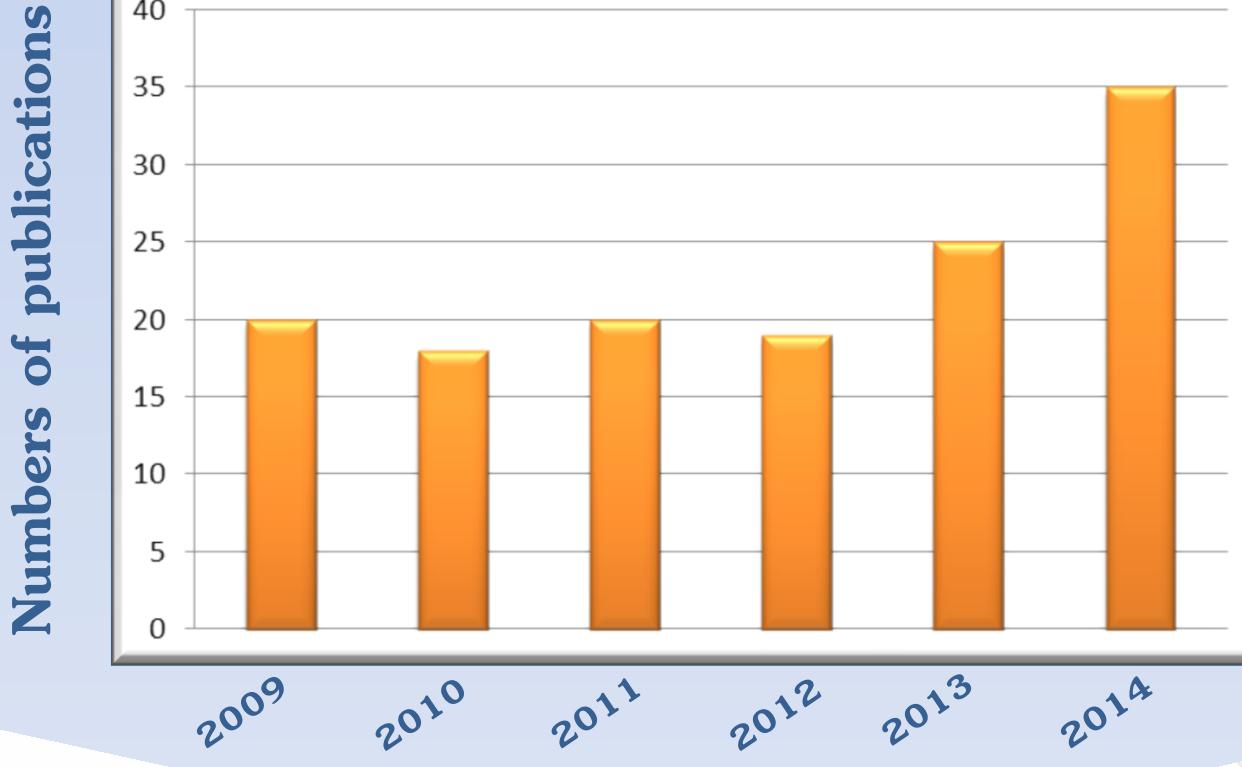
Infected with Py pRBC



# โครงการวิจัย/แหล่งทุน



## Publications in International Journals



*Impaired TLR5 Functionality Is Associated with Melioidosis*

T. Eoin West,<sup>a,c</sup> Narisara Chanratita,<sup>b,d</sup> Wirun  
Dirék Liummathuretsakul,<sup>b,d</sup> Vanan  
Mary J. Emond,<sup>e</sup> Mackenzie  
Shawn J. Stroh<sup>f</sup>

*The American Journal of Tropical Medicine and Hygiene*, Vol. 80, No. 5, May 2009  
 doi:10.4299/ajtmh.08-0172  
 Copyright © 2009 by The American Society of Tropical Medicine and Hygiene

**Short Report: Rapid Detection of *Burkholderia pseudomallei* in Blood Cultures Using a Monoclonal Antibody-Based Immunofluorescent Assay**

Narinsa Chantawannakul,<sup>1</sup> Sarunpong Tanchararak,<sup>1</sup> Gunphol Wongwaiyai,<sup>1</sup> Vanaporn Wuthikarn,<sup>1</sup> Nitaya Teerawattananon,<sup>1</sup> Nicholas P. J. Day,<sup>2</sup> Drisit Limmathurotsakul,<sup>1</sup> and Sharon J. Peacock<sup>3</sup>  
*Department of Microbiology and Immunology, Mahidol-Oxford Tropical Medicine Research Unit,<sup>1</sup> and Department of Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Department of Clinical Pathology, Sappaphrayaeng Hospital, Ubon Ratchathani, Thailand; The Wellcome Trust Clinical Research Facility, Department of Clinical Medicine, University of Oxford, Churchill Hospital, Oxford, United Kingdom; Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Cambridge, United Kingdom*

**Abstract.** Melioidosis is a severe bacterial infection caused by *Burkholderia pseudomallei*. Rapid antimicrobial therapy is necessary to improve rates of outcome, which is aided by direct detection of *B. pseudomallei* in clinical samples. A drawback for all antigen assays is that the number of *B. pseudomallei* in blood usually falls below the achievable level of detection. We performed a prospective cohort study of 461 patients with 541 blood cultures to evaluate the utility of a modified agglutination assay (Math-IFA) for the detection of *B. pseudomallei* in blood cultures. The Math-IFA used immunochromatographic antibody-based detection of *B. pseudomallei* flagellin and capsular polysaccharide antigen. Of the 461 patients, 174 (37.8%) were culture positive for *B. pseudomallei* and 286 (62.2%) were culture negative. Of those who did not have a blood culture containing *B. pseudomallei* (specificity = 100%), the Math-IFA could detect 265 patients (92.7%). The Math-IFA could be a valuable supplementary tool for rapid detection. We recommend the use of the Math-IFA to test blood cultures that flag positive in regions where melioidosis is endemic.

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**ARCH ARTICLE** |  
Role of short-chain dehydrogenase/reductase, induced by salt stress, in interaction of *B. pseudomallei* with *Sphaerotilus* sp. and *Leptothrix* sp.

Society and Democracy 2018, 5(3)  
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**The In-vitro Antibacterial Effects of Colored Rose Crude Extracts against Staphylococcus aureus** *Evaluation with Skin and Soft Tissue Infection*

Environ Biol Fish (2013) 98:1–10  
DOI 10.1007/s10641-012-0103-0  
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**Abstract** The present study was conducted to evaluate the antibacterial activity of colored rose extracts against *S. aureus*, which is one of the most important causative agents of skin and soft tissue infections. The extracts were prepared from the petals of three different colored roses (red, white, and yellow) and their antibacterial activity was evaluated by the disk diffusion method. The results showed that all three extracts had inhibitory effects on *S. aureus*. The inhibition zone diameter increased with increasing concentration of the extracts. The red rose extract was found to be more effective than the other two extracts. The minimum inhibitory concentration (MIC) of the red rose extract was determined as 100 mg/ml. The MIC values of the white and yellow rose extracts were 200 and 300 mg/ml, respectively. The results of this study indicated that the colored rose extracts have potential antibacterial properties and may be used as a natural source for the development of new antimicrobial agents.

**Keywords** *S. aureus* • Rose • Antibacterial activity • Colored rose extract

**Introduction** *Staphylococcus aureus* is a Gram-positive bacterium that is widely distributed in nature and can cause various diseases in humans and animals [1]. *S. aureus* is a causative agent of many diseases such as food poisoning, toxic shock syndrome, necrotizing fasciitis, and skin and soft tissue infections [2]. *S. aureus* is a major causative agent of skin and soft tissue infections [3].

**Materials and Methods** **Microorganism** *S. aureus* (ATCC 25923) was obtained from the National Collection of Type Cultures (London, UK).

**Antibacterial Assay** The antibacterial activity of the extracts was evaluated by the disk diffusion method. The bacterial culture was prepared by inoculating 1 ml of the bacterial suspension (10<sup>6</sup> CFU/ml) into 10 ml of Mueller-Hinton Broth (Becton Dickinson, Franklin Lakes, NJ, USA). After 24 h of incubation at 35 °C, 10 µl of the culture was applied onto the surface of Mueller-Hinton Agar (Becton Dickinson) plates. The agar plates were dried at room temperature for 1 h and then 10 µl of each extract was applied onto the surface of the agar plates. The agar plates were dried again at room temperature for 1 h and then the antibiotic disks (Oncor, Gaithersburg, MD, USA) were placed onto the agar plates. The antibiotic disks contained 30 µg of ciprofloxacin as the positive control and 10 µg of kanamycin as the negative control. The agar plates were incubated at 35 °C for 24 h and the inhibition zones were measured.

**Determination of Minimum Inhibitory Concentration (MIC)** The MIC of the extracts was determined by the serial dilution method. The bacterial culture was prepared by inoculating 1 ml of the bacterial suspension (10<sup>6</sup> CFU/ml) into 10 ml of Mueller-Hinton Broth (Becton Dickinson). After 24 h of incubation at 35 °C, 10 µl of the culture was applied onto the surface of Mueller-Hinton Agar (Becton Dickinson) plates. The agar plates were dried at room temperature for 1 h and then 10 µl of each extract was applied onto the surface of the agar plates. The agar plates were dried again at room temperature for 1 h and then the antibiotic disks (Oncor, Gaithersburg, MD, USA) were placed onto the agar plates. The antibiotic disks contained 30 µg of ciprofloxacin as the positive control and 10 µg of kanamycin as the negative control. The agar plates were incubated at 35 °C for 24 h and the inhibition zones were measured.

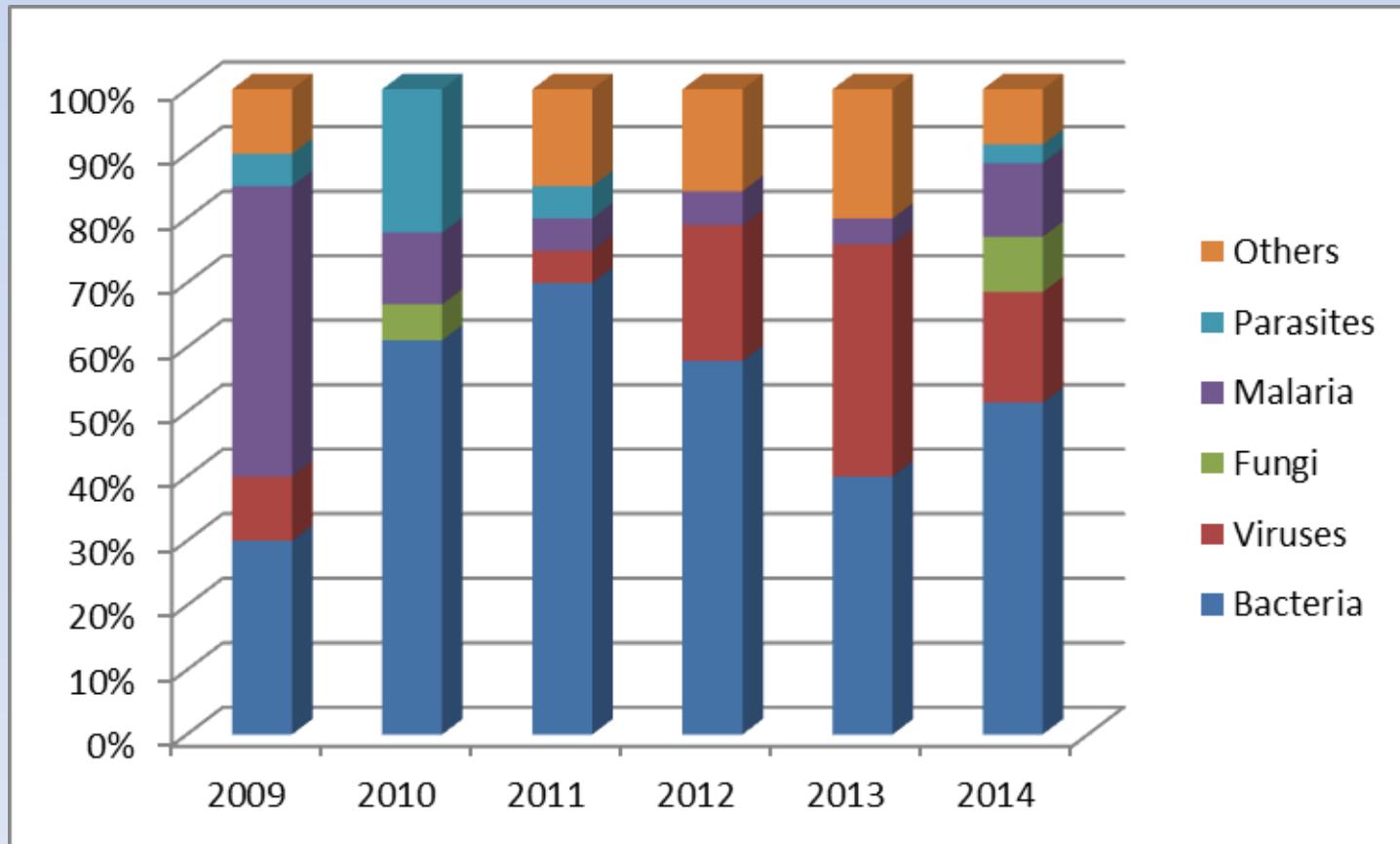
**Statistical Analysis** The statistical significance of the differences between the mean values was calculated by one-way analysis of variance (ANOVA) and Tukey's HSD test.

**Results** The results of the antibacterial activity of the extracts are shown in Table 1. The inhibition zone diameter increased with increasing concentration of the extracts. The red rose extract was found to be more effective than the other two extracts. The MIC of the red rose extract was determined as 100 mg/ml. The MIC values of the white and yellow rose extracts were 200 and 300 mg/ml, respectively.

**Discussion** The results of this study indicated that the colored rose extracts have potential antibacterial properties and may be used as a natural source for the development of new antimicrobial agents.

**Conclusion** The results of this study indicated that the colored rose extracts have potential antibacterial properties and may be used as a natural source for the development of new antimicrobial agents.

# Publications during 2009 to 2014

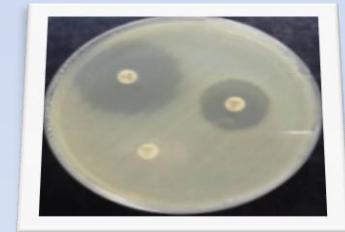


# Clinical Service

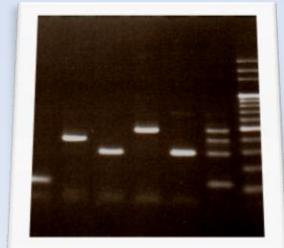
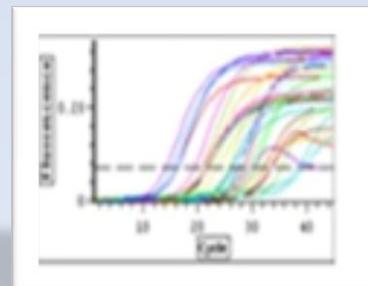


## Conventional cultivation for aerobic bacteria and reagents for biochemical tests

(บริการตรวจวิเคราะห์เชื้อแบคทีเรียแบบ ไข้ออกซิเจน เชื้อรา และผลิตัวสตั๊ดสำหรับ  
การตรวจและการสอนทางจุลชีววิทยา)



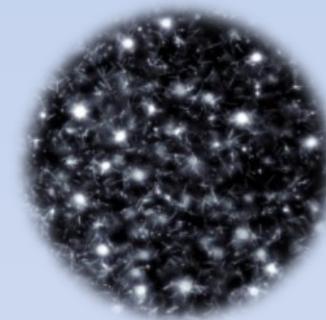
Detection of viral infections by Real-time RT-PCR,  
RT-PCR, Nested RT-PCR,



# Immunodiagnosis

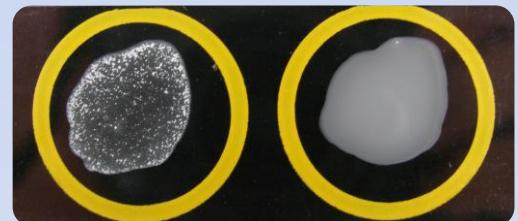
1. Detection of antibody against *Leptospira* spp.

Microscopic Agglutination Test (MAT)



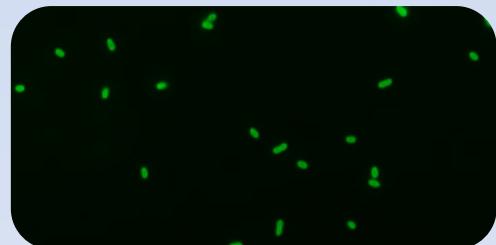
2. Detection of *Burkholderia pseudomallei*

by latex agglutination



3. Detection of *Burkholderia pseudomallei*

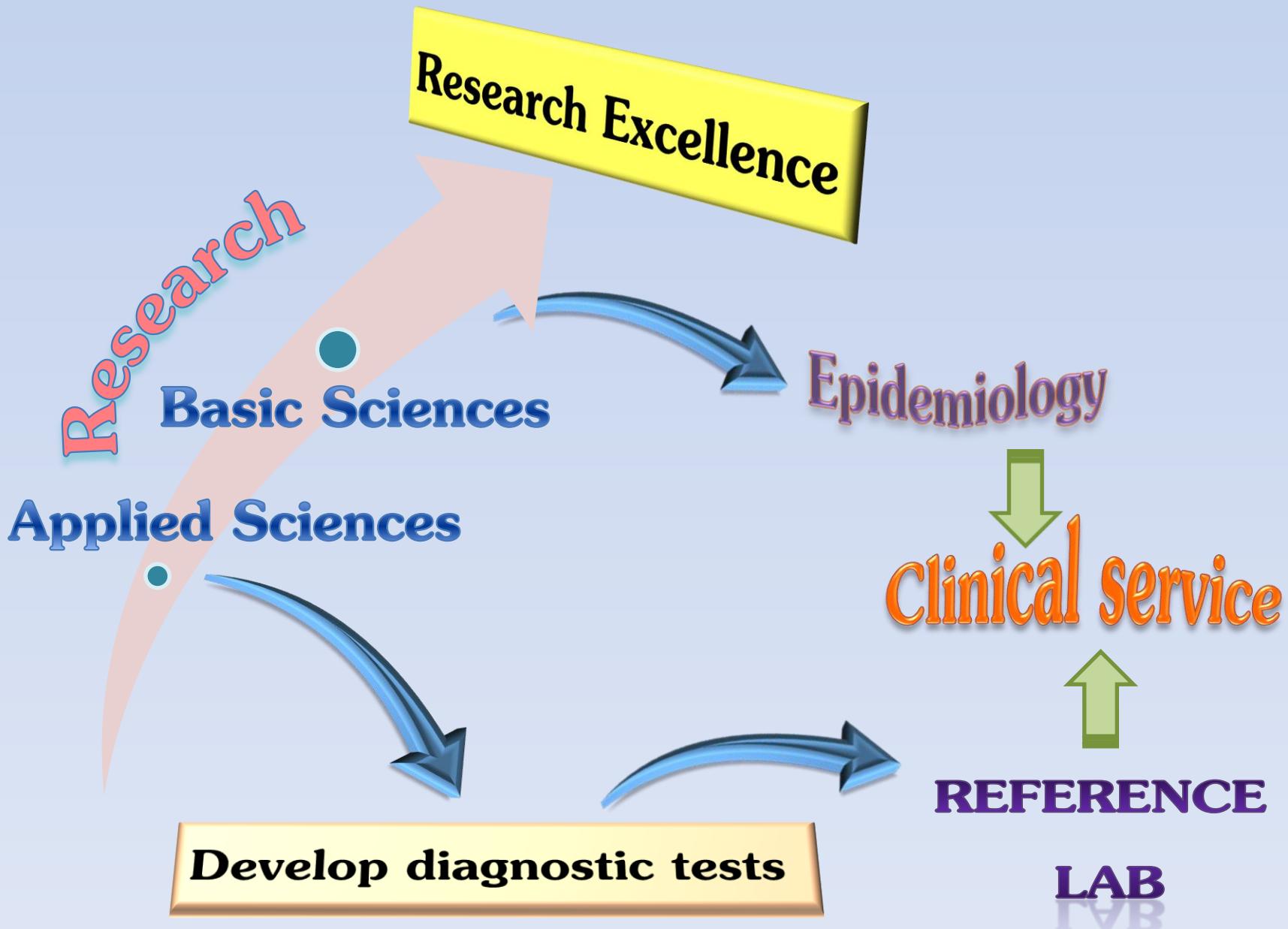
by Immunofluorescence Assay



4. Detection of viral - specific antibodies by ELISA

5. Determination of CD4/CD8 T cell population by Flow

Cytometry



**Strength** →



**Opportunity**



**Weakness**



**Threat**



**NO SEVERE  
THREAT**



# ขอขอบคุณ



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**Thank you for your  
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