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TMID 607-16:

Molecular aspects in food- and water-borne diseases II

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TMID607-16: Learning Objectives

1) Describe strategies to control Foodborne (FB) and Waterborne (WB) diseases.
2) Describe advance molecular approaches for tracking (AMR) pathogens, public health surveillance, and outbreak.

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TMID607-16: Learning Objectives

3) Integrate -omics technology for developing a novel molecular diagnostic and biopharmaceutic reagent for emerging FB and WB diseases outbreak.

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Learning Evaluation

- Essay-type Examination
- MEQ

Learning Resource

1) PowerPoint slide presentation
2) Handout
3) Textbooks
4) Suggested readings

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Textbook:

- Fratamico P, Liu Y, Kathariou S, editors. Genomes of foodborne and waterborne pathogens. ASM Press American Society for Microbiology Washington, DC; 2011.

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Textbook:

- Barh D, Blum K, and Madigan MA, editors. OMICS Biomedical Prespectives and Applications. CRC Press Tayler & Francis Group Boca Raton; 2012.

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Suggested readings:

1) Hashempour-Baltork F, Hosseini F, Shojaee-Aliabadi S, Torbati M, Alizadeh AM, Alizadeh M. resistance and the prevention strategies in food borne bacteria: an update review. *Adv Pharm Bull.* 2019; 9(3): 335-347.

2) Zhao X, Lin CW, Wang J, and Oh DH. Advances in rapid detection methods for foodborne pathogens. *J Microbiol Biotechnol.* 2014; 24(3): 297–312.

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Suggested readings:

3) Maneewatchararangsri S. Therapeutic monoclonal antibodies and their engineered antibody fragments specific to LipL32 for passive immunotherapy of leptospirosis. *J Virol Emerg Dis* 2016; 2(2). Available from: doi http://dx.doi.org/10.16966/ 2473-1846.114.

4) Maneewatch S, Thanongsaksrikul J, Songserm T, Thueng-In K, Kulkeaw K, Thathaisong U, et al. Human single-chain antibodies that neutralize homologous and heterologous strains and clades of influenza A virus subtype H5N1. *Antivir Ther.* 2009; 14(2): 221-30.

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Suggested readings:

5) Maneewatch S, Sakolvaree Y, Tapchaisri P, Saengjaruk P, Songserm T, Wongratanacheewin S. Humanized-monoclonal antibody against heterologous *Leptospira* infection. *Protein Eng Des Sel.* 2009; 22(5): 305-312.

6) Thanongsaksrikul J, Srimanote P, Maneewatch S, Choowongkomon K, Tapchaisri P, Makino S, et al. A V_HH that neutralizes the zinc-metalloproteinase activity of botulinum neurotoxin type A. *J Biol Chem.* 2010; 285(13):9657-66.

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Outline:

1. Summary of foodborne (FB) and waterborne (WB) diseases
2. Impacts of FB and WB diseases
3. Strategies to control FB and WB diseases
4. Emergence of antimicrobial resistant (AMR) pathogens & control
5. Current development of therapeutic antibodies for FB and WB passive therapy

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Outline:

1. Summary of foodborne (FB) and waterborne (WB) diseases

- Waterborne & Waterborne disease
- Key foodborne disease-causing organisms and disease

2. Impacts of FB and WB diseases

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Foodborne Disease & Food poisoning:

Caused by ingested food contaminated with microorganisms, such as bacteria, fungi, viruses, parasites and by natural toxins, chemical and physical agents which tend to have acute effects on human health.

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Water-borne diseases

- Diseases caused by ingestion of water contaminated by human or animal excrement, which contain pathogenic microorganisms.
- Including cholera, typhoid, amoebic and bacillary dysentery and other diarrheal diseases

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Water-related diseases

- Caused by insect vectors, e.g. mosquitoes, that breed and feed near contaminated water.
- Lack of access to clean drinking water or sanitation services.
- Examples: dengue, filariasis, malaria, onchocerciasis, trypanosomiasis, yellow fever.

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Water-washed diseases

- Caused by poor personal hygiene and skin and eye contact with contaminated water
- Include scabies, trachoma, typhus, other flea, lice, and tick-borne diseases.

Water-based diseases

- Caused by parasites found in intermediate organisms

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Foodborne Disease & Food poisoning:

Bacteria

- *Bacillus cereus*
- *Brucella spp.*
- *Campylobacter jejuni*
- *Clostridium botulinum*
- *Clostridium perfringens*
- *Escherichia coli*
- *Salmonellosis*
- *Shigella spp.*
- *Staphylococcus aureus*
- *Vibrio parahaemolyticus*
- *Vibrio cholerae*

Virus

- **Hepatitis A**
- **Norovirus**
- **Rotavirus**

Protozoa

- *Entamoeba histolytica*
- *Giardia lamblia*

Toxin & Chemicals

- **Insecticide**
- **Poisonous plant**
- **Poisonous seafood (Seafood intoxication)**

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Diseases related to water

Water-Borne Disease
- Cholera
- Typhoid
- Amoebic and bacillary dysentery
- Diarrheal disease

Water-Washed Disease
- Scabies
- Trachoma
- Flea,
- lice and tick-borne diseases

Water-based Disease
- Schistosomiasis
- Dracunculiasis

Water-Related Insect-Vector Disease
- Filariasis, Malaria
- Onchocerciasis,
- Trypanosomiasis
- Yellow fever

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Foodborne parasites=FBP

Common parasites:
Trichinellosis
Opisthorchiasis
Cryptosporidiosis

Helminth: liver and intestinal flukes
Fasciola spp.
Paragonimus spp.
Echinococcus spp.
Taenia spp.
Angiostrongylus spp.
Ascaris spp.
Toxocara spp.
Trichinella spp.

Protozoa:
Cryptosporidium spp.,
Cyclospora cayetanensis,
Toxoplasma gondii

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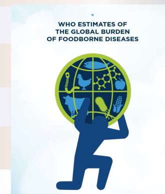
Key foodborne disease-causing organisms and disease (WHO, 2010)

Hazard	Disease
DIARRHOEAL DISEASE AGENTS	
Campylobacter spp.	Diarrhoeal disease/ Guillian-Barre syndrome
Enteropathogenic *Escherichia coli*	Diarrhoeal disease
Enterotoxigenic *E. coli*	Diarrhoeal disease
Shiga toxin-producing *E. coli*	Diarrhoeal disease
Noroviruses	Diarrhoeal disease
Non-typhoidal *S. enterica*	Diarrhoeal disease/ Invasive salmonellosis
Shigella spp.	Diarrhoeal disease
Vibrio cholerae	Diarrhoeal disease
Entamoeba histolytica	Diarrhoeal disease
Giardia spp.	Diarrhoeal disease



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Key foodborne disease-causing organisms and disease (WHO, 2010)

Hazard	Disease
INVASIVE INFECTIOUS DISEASE AGENTS	
Hepatitis A virus	Hepatitis
Brucella spp.	Acute brucellosis/ Chronic brucellosis/ Orchitis
Listeria monocytogenes, perinatal	Sepsis/ CNS infection/ Neurological sequelae
Listeria monocytogenes, acquired	Sepsis/ CNS infection/ Neurological sequelae
Mycobacterium bovis	Tuberculosis
Salmonella Paratyphi	Paratyphoid fever/ Liver abscesses and cysts
Salmonella Typhi	Typhoid fever/ Liver abscesses and cysts
Toxoplasma gondii, congenital	Intracranial calcification/ Hydrocephalus Chorioretinitis, early in life, later in life CNS abnormalities

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Key foodborne disease-causing organisms and disease (WHO, 2010)

Hazard	Disease
Toxoplasma gondii, acquired	Chorioretinitis, mild, moderate, severe Acute illness/ Post-acute illness
ENTERIC INTOXICATIONS	
Bacillus cereus	Acute intoxication
Clostridium botulinum	Moderate/ mild botulism/ Severe Botulism
Clostridium perfringen	Acute intoxication
Staphylococcus aureus	Acute intoxication
Trichinella spp.	Acute clinical trichinellosis

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Key foodborne disease-causing organisms and disease (WHO, 2010)

Hazard	Disease
CESTODES	
Echinococcus granulosus, causes seeking treatment	Pulmonary cystic echinococcosis Hepatis cystic echinococcus CNS cystic echinococcus
Echinococcus granulosus, causes not seeking treatment	Pulmonary cystic echinococcosis Hepatis cystic echinococcus CNS cystic echinococcus
Echinococcus multilocularis	Alveolar echinococcosis
Taenia solium	Epilepsy: treated, seizure free Epilepsy, treated with recent seizures
NEMATODES	
Ascaris spp.	Ascariasis infestation Mild abdominopelvic problems
Trichinella spp.	Acute clinical trichinellosis

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Key foodborne disease-causing organisms and disease (WHO, 2010)

Hazard	Disease
TREMATODES	
Clonorchis sinensis	Abdominopelvic problems due to heavy clonorchiosis
Fasciola spp.	Abdominopelvic problems due to heavy fasciolosis
Intertinal flukes	Abdominopelvic problems due to heavy intestinal fluke infections
Opisthorchis spp.	Abdominopelvic problems due to heavy opisthorchiosis
Paragonimus spp.	Abdominopelvic problems due to heavy paragonimiosis Pulmonary problems due to heavy paragonimosis

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Impact of foodborne & waterborne diseases

- A global public health burden, cause morbidity and mortality worldwide.
- Consumption of food or drink contaminated with pathogens or toxins.

- **Human**
- **Animals**
- **Economy**

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The global burden of foodborne disease

Citation: World Health Organization, *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015*. 2010

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Incidence of foodborne diseases

The burden of **foodborne diseases** is substantial

Every year foodborne diseases cause:

almost **1 In 10** People to fall ill

33 million Healthy life years lost

Foodborne diseases can be deadly, especially in Children <5

420 000 death

Children account for **1/3** of death from food diseases

FOODBORNE DISEASES ARE PREVENTABLE.
EVERYONE HAS A ROLE TO PLAY.

WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015.

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Incidence of waterborne diseases

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Every year

> 100 million People to fall ill

37 000 die

32 million Children <5 fall ill

Diarroeal diseases are responsible for 70% of the burden of foodborne diseases

E. coli Norovirus Campylobacter Non-typhoidal *Salmonella*

WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015.

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The global burden of foodborne disease by subregion caused by enteric hazard, 2010.

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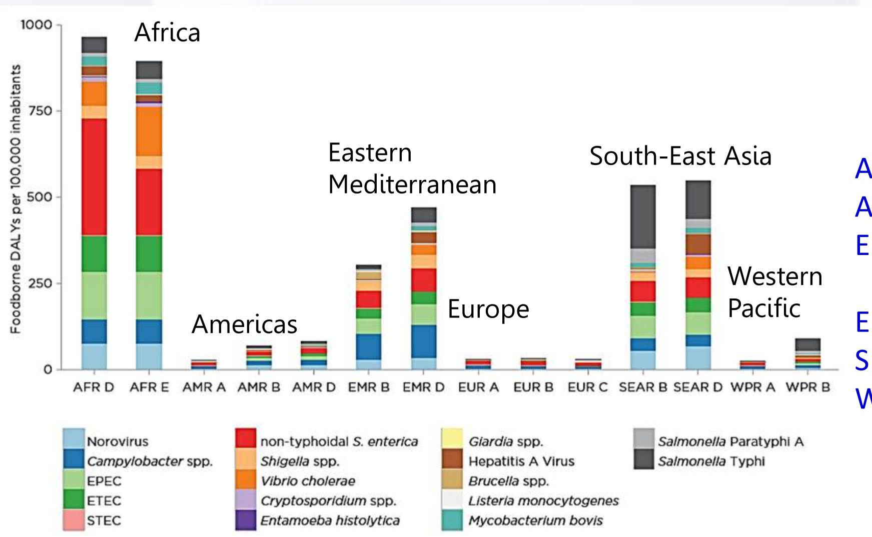

AFR	Africa
AMR	Americas
EMR	Eastern-Mediterranean
EUR	Europe
SEAR	South-East-Asia
WPR	Western Pacific

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The global burden of foodborne disease by subregion caused by hazard groups, 2010.

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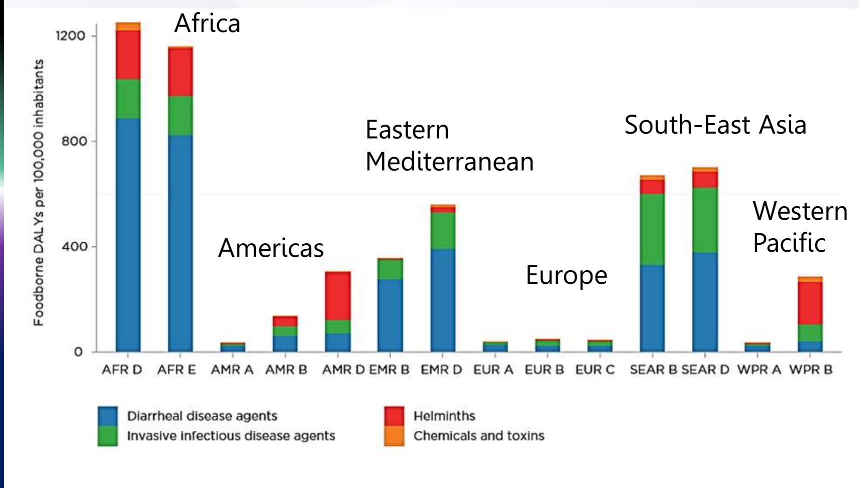

AFR	Africa
AMR	Americas
EMR	Eastern-Mediterranean
EUR	Europe
SEAR	South-East-Asia
WPR	Western Pacific

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HOSPITAL FOR TROPICAL DISEASES MAHIDOL UNIVERSITY

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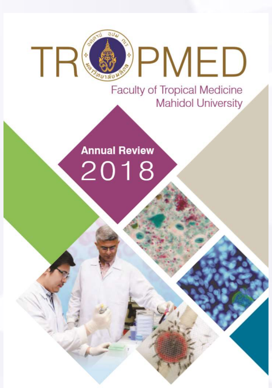

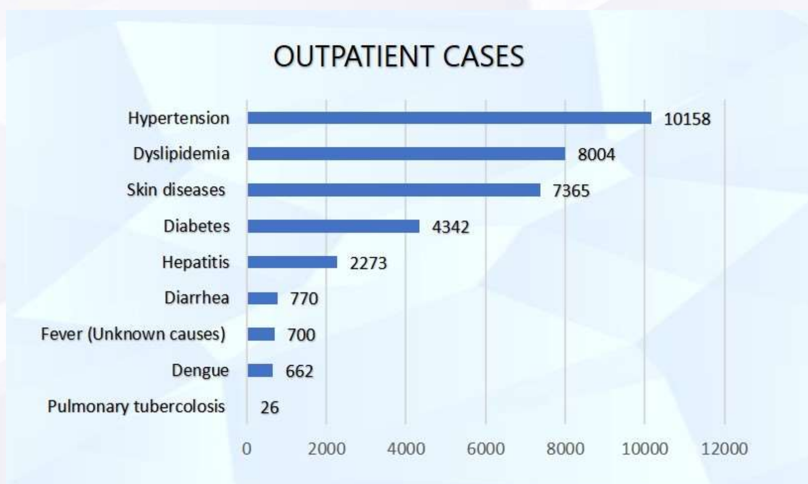

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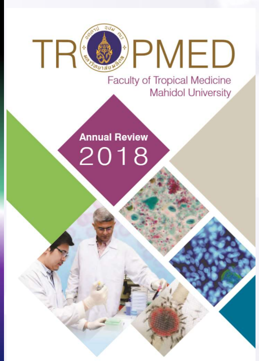

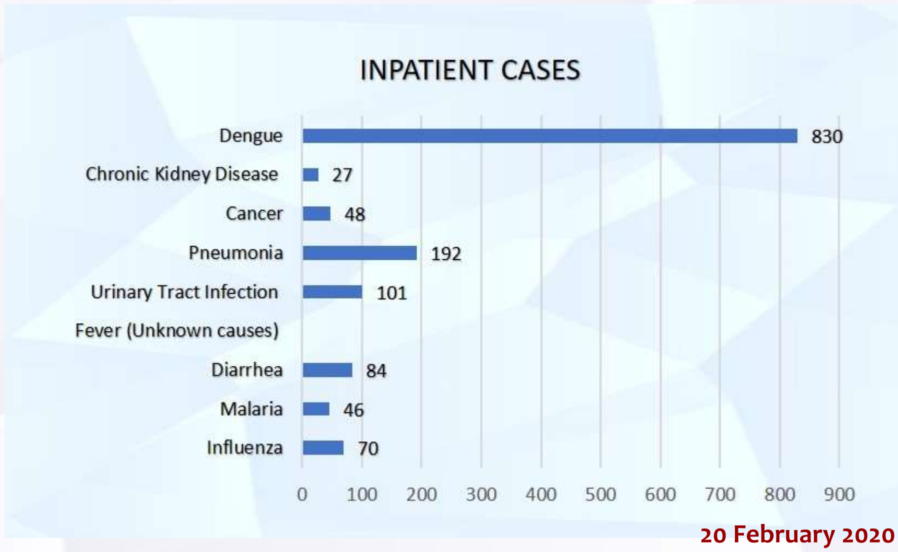

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Outline:

3. Strategies to control FB and WB diseases

- Strategies to control disease
- Molecular approaches for FB and WB diseases

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The need for foodborne diseases: to preventing and controlling outbreak

- Improved technologies for foodborne diseases surveillance, and diagnosis
- Strong policies and investment in training and infrastructure
- Need public-private partnerships supporting food safety
- Early detection and response to foodborne outbreaks
- Effective control measures

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Strategies to control FB and WB diseases

- Precise identify and tracking disease
- Strengthening food safety
- Surveillance

SURVEILLANCE

FOOD SAFETY

ADVANCED MOLECULAR DETECTION

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Surveillance system

- Trace-back & Epidemiological investigation
- Improved foodborne outbreak detection and investigation
- Improved surveillance of rare foodborne infections
- Assessment of contamination patterns of food industry

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Action plan

- Foodborne disease surveillance programme:
  to improve safety of food supply and prevent foodborne infections.
- Early screening test: development of rapid testing methods

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Strengthening Food Safety

- CDC helping PulseNet laboratories throughout USA
- Connect foodborne illnesses to detect and control outbreak

As of March 2019, 69 laboratories in 49 states were **PulseNet Lab** certified for whole genome sequencing (WGS) of 4 foodborne bacteria*: Salmonella, Listeria, E. coli and Campylobacter.*

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Strengthening Food Safety

PulseNet Lab*: Salmonella, Listeria, E. coli and Campylobacter.*

○ PulseNet Labs
● PulseNet Labs certified for WGS as of Dec 17
★ Area Labs All are certified for WGS

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PulseNet: USA
the national laboratory network for foodborne outbreak detection

FoodNet: USA
the foodborne Disease Active Surveillance Network

TraNet: China
a national foodborne disease molecular tracing network

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TraNet: a national foodborne disease molecular tracing network

- Surveillance of foodborne bacteria from human ,food, environment
- Collects molecular subtyping databases of foodborne pathogens from a geographically diversity to facilitate early detection of outbreaks

Weiwei et al. (2018)

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TraNet: a national foodborne disease molecular tracing network

- Genotyping (DNA fingerprint) based on pulsed-field gel electrophoresis (PFGE) patterns of isolates
- Whole genome sequencing (WGS) technology
- Serotyping data
- AMR data and pathogenicity (Virulence profile)

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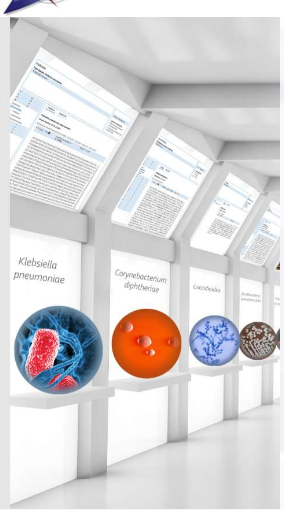
MicrobeNet



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MicrobeNet and Advanced Molecular Detection (AMD):

- Reference laboratory for emerging bacteria and fungi
- Reduce time to identify a pathogen from days to hours
- AMD support and in collaboration with industry partners

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Conventional Detection Methods

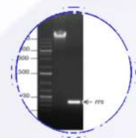
- Direct examination
- Culture-based methods
- Immunological-based methods
- Polymerase chain reaction


Direct examination


CULTURE


IgM-/ IgG-ELISA


Immuno-fluorescence (IFA)


Molecular diagnosis

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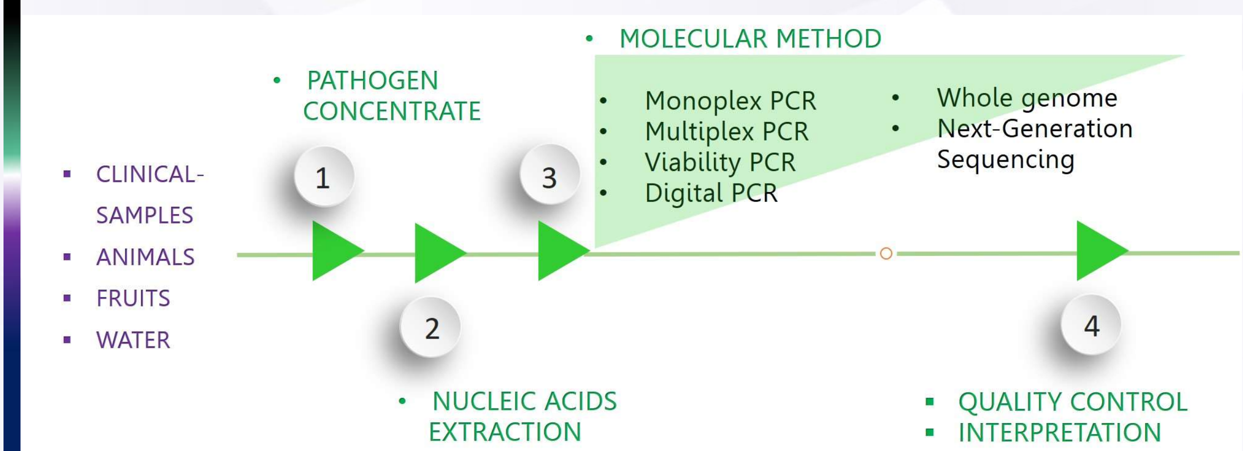
Workflow of molecular detection and characterization of food- and water-borne pathogens



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Comparison PCR and high technologies in detection and characterization of food- and water-borne pathogens I

Monoplex RT qPCR
- widely used
- validated & in use in European

Viability PCR
- risk assessment

Multiplex RT qPCR
- High cost reduction
- Slight loss of sensitivity
- Cost-effective for surveillance studies

Adapted from : BVosch A, Pinto RM, and Guix S. Foodborne viruses. Current Opinion in Food Science. 2016; 8: 110-119. Doi: http://dx.doi.org/10.1016/j.cofs.2016.04.002.

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Comparison PCR and high technologies in detection and characterization of food- and water-borne pathogens II

Digital RT dPCR
- Quantitative data without standard curve
- Simultaneous quantification of several targets

Whole Genome Sequencing (WGS)

Next-Gen Sequencing (NGS)
- Technically challenging
- Biotracking
- Environmental viral diversity

Adapted from : BVosch A, Pinto RM, and Guix S. Foodborne viruses. Current Opinion in Food Science. 2016; 8: 110-119. Doi: http://dx.doi.org/10.1016/j.cofs.2016.04.002

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Technological advances improving foodborne illness surveillance and Detection

- Molecular subtyping technology: PFGE, WGS
- Identify pathogens in food products and clinical samples: MALDI-TOF MS
- Biosensor: CMOS microdevices
- Nucleic acid amplification methods: PCR, RT-PCR, LAMP
- Point of care (POCT) diagnostics

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INNOVATION

Whole genome sequencing (WGS)

In 2017, CDC used WGS to:

- Confirm campylobacter infection.
- Tracking source of pathogens, and outbreak.
- Analyze antimicrobial resistant genes.

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INNOVATION

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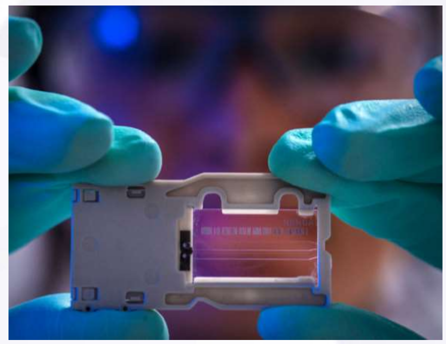
Whole genome sequencing (WGS)

Rapid and precise identification of the bacteria causing foodborne illness is critical for timely foodborne outbreak response.

GIVES AN EXACT DNA PROFILE
OF FOODBORNE PATHOGENS
& OUTBREAKS.



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Next-Generation sequencing: Neuroleptospirosis diagnosis

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-Generation sequencing. *N Eng J Med*. 2014; 370: 2408-2417. Doi:10.1056/NEJMoa1401268.

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Neuroleptospirosis diagnosis

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Diagnosis of *Leptospira* infection by Means of Unbiased Next-Generation Sequencing (NGS)

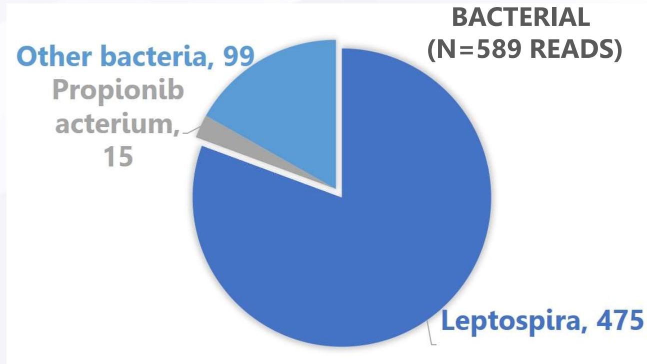
Start
Aug 21, 2013

NGS library generation

NGS library validation

NGS analysis

End
Aug 23, 2013



Wilson *et al.*, 2014

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Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

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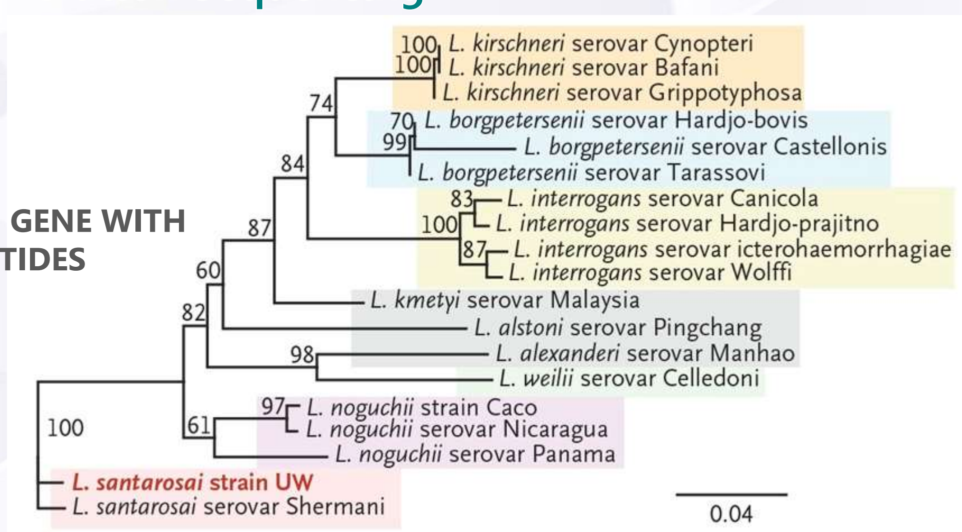
rpoB

FULL-LENGTH GENE WITH 3681 NUCLEOTIDES



Wilson *et al.*, 2014

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(Next-Next) Generation Sequencing

Single-molecule sequencing (SMS) technology

HeliScope™ Single Molecule Sequencer

-Analysis of human genomic without cloning, amplification and ligation processes.

-Personal genomics (Pushkavev *et al.*, 2009)

Citation: Pushkarev D, Neff NF, and Quake SR. Single-molecule sequencing of an individual human genome. Nat Biotechnol. 2009. 27(9):847-50. doi: 10.1038/nbt.1561.

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Single-molecule sequencing (SMS) technology

SMRT® Single molecule, real-time Technology
-Two key innovations, Zero-Mode Waveguides and Phospholinked Nucleotides

Application:

- Generate microbial genomes
- Analyze genome-wide methylation with single-base resolution
- Detect and resolve plasmids, mobile elements, and structural variation including gene duplication and inversion.

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Mass Spectrometry (MS)

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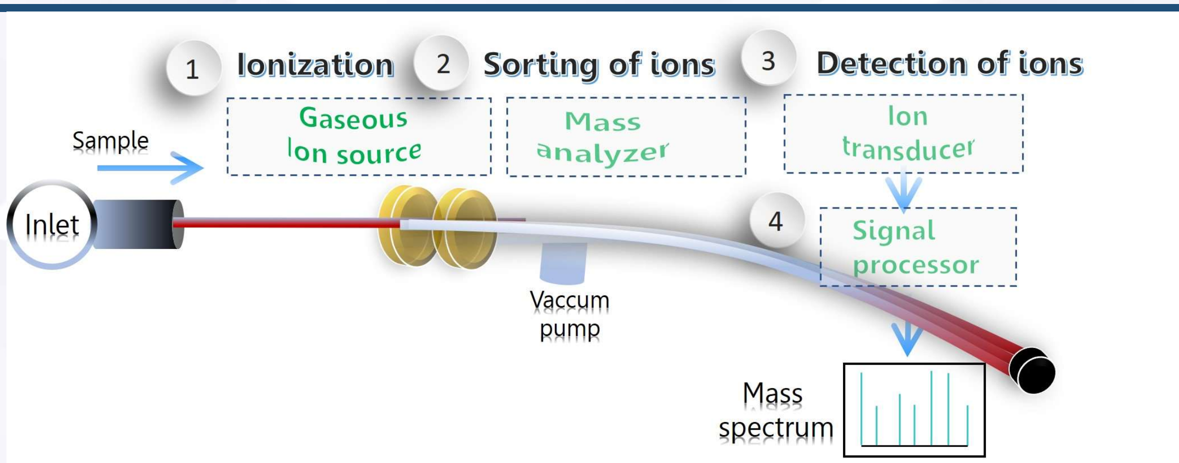
- Mass Spectrometry (MS) measures the atomic or molecular weight of a ion from the separation based on its mass to charge ratio (*m/z*)



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Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS)

- rapid, sensitive to identify microorganisms
- Limited by protein database available
- Six common foodborne pathogens:

  Shigella flexneri, Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritidis

Ref: Wenjing Y *et al.* (2020)

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Diagnostics 4.0:
Leptospira detection & Identification by MALDI-TOF MS

Whole cell matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) for identification of *Leptospira* spp. in Thailand and Lao PDR.

Sonthayanon P, Jaresitthikunchai J, Mangmee S, Thiangtrongjit T, Wuthiekanun V, Amornchai P, et al. Whole cell matrix assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of Leptospirosis spp. in Thailand and Lao PDR. *PloS Negl Trop Dis.* 2019; 13(4):e0007232.

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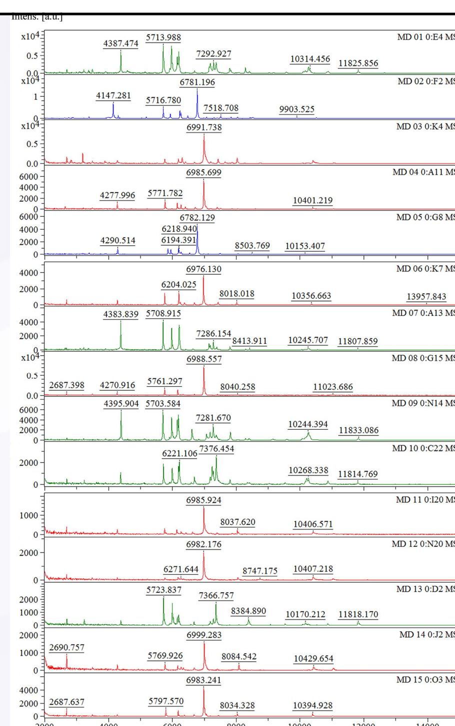
Identify *Leptospira* based on MALDI-TOF MS

Differentiation of *L. interrogans*, *L.kirschneri*, and *L. borgpetersenii* based on MALDI-MS algorithm

Sonthayanon *et al.*, 2019



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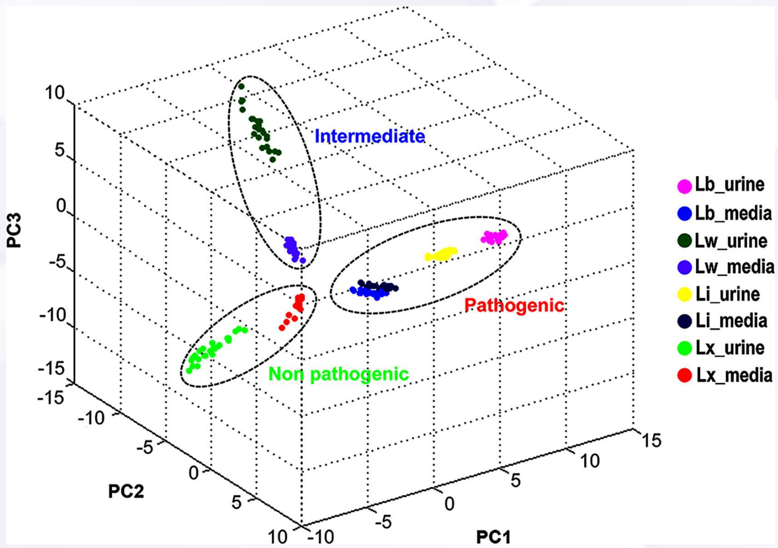
Principle component analysis (PCA) of protein spectra from spiked leptospires in urine and media.

Each dot displayed a protein spectrum of each sample.

L. borgpetersenii (Lb) (Lb)
L. wolffii (Lw) (Lw)
L. biflexa (Lx) (Lx)
L. interrogans (Li) (Li)



Sonthayanon *et al.*, 2019

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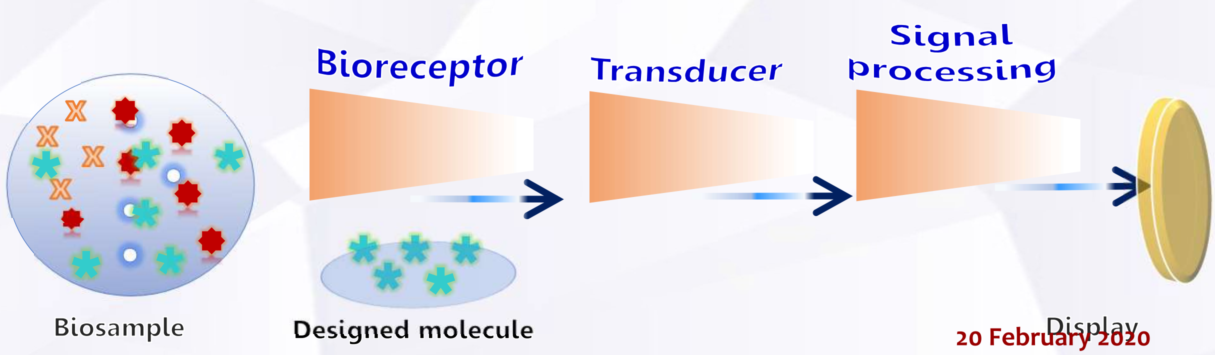
Biosensor

A biosensor is an analytical device which is used to determine the presence and concentration of a specific substance in a biological analyte



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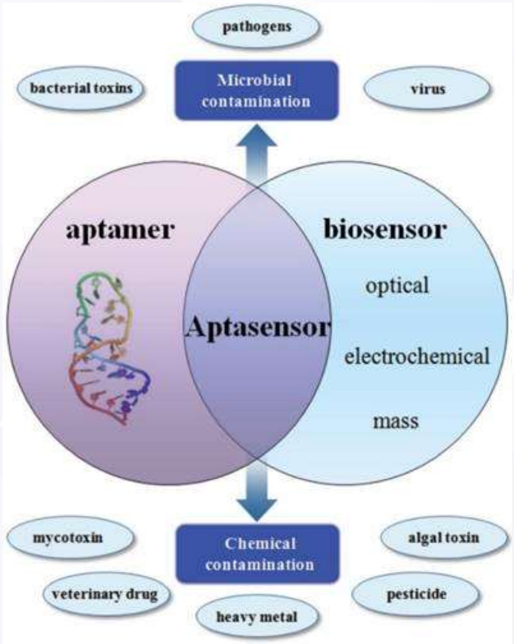

Aptasensor using aptamers to detect nucleotides and peptide of FB pathogens

Citation: Neethirajan S, Ahmed SR, Chand R, Buozis J, Nagy E. Recent advances in biosensor development for foodborne virus detection. *Nanotheranostics.* 2017. 1(3): 272-295. Doi: 10.7150/ntno.20301.

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ADVANCED MOLECULAR DETECTION

Advanced Molecular Detection (AMD)

- **Precise identifying and tracking a global health diseases:**
  - -identify and track (emerging) pathogens fast,
  - -providing strain sub-typing,
  - -characterize antimicrobial resistance (AMR)
- **Data sharing in the cloud**
- **Safe from illness & stop outbreak sooner, control disease**

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ADVANCED MOLECULAR DETECTION

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Perspective
Advanced Molecular Detection (AMD)

- **Hand-held sequencers for field investigations**
- **Next-generation genomic sequencing technologies:**
  WGS, NGS, nanopore sequencing
- **High performance computing systems:**
  Cloud

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ADVANCED MOLECULAR DETECTION

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CDC' AMD program: develops rapid tests for:

-Infectious diseases

-Foodborne diseases

-Influenza

-Tuberculosis

-Malaria

-Parasitic diseases

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Next-Generation Point-of-Care Testing (POCT): Molecular platform

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"FeverPhone" Smartphone based molecular diagnostics platform for point-of-care differential diagnosis of six common causes of acute febrile illness.

- **Leptospirosis**
- **Dengue**
- **Chaga**
- **Typhoid**
- **Chikungunya**
- **Malaria**

Citation: David Carl E, Saurabh M. FeverPhone: *Point of care diagnosis of acute febrile illness using a mobile device.* Cornell University. 2017.

65

Next-Generation Point-of-Care Testing (POCT): Molecular platform

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BioMeme's
Handheld PCR

The Philadelphia-based startup Biomeme has developed a real-time PCR thermocycler that attaches to an iPhone, enabling the user to perform gold-standard DNA analysis in the field.

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Next-Generation Point-of-Care Testing (POCT): Molecular analyzer

Alere q: automated nucleic acid testing platform (PCR) with multiplexing capability that enables point-of-care access to a suite of molecular assays, bypassing issues with centralized testing and reducing patient loss to follow up.

Alere
Rapid point-of-care diagnostics.

Citation: Hsaiao NY, Dunning L, Kroon M, Myer L. Laboratory evaluation of the Alere q point-of-care system for early infant HIV diagnosis. *PLoS One*. 2016; 11(3):e0152672.

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Driving of eHealth growth

Diagnostics 4.0: e-Health Diagnostics

- Internet of Things (IoT)
- Robotics
- Artificial Intelligence
- Advanced Virtual Reality
- Space Colonization
- 3D Printing
- Medical Innovation
- High-Speed Travel
- Blockchain Technology
- Autonomous Vehicles
- Renewable Energy

Citation: Ministry of Public Health, *eHealth Strategy, Ministry of Public Health (2017– 2026).* August 2017. Available from https://ehealth.moph.go.th [Accessed 27 May 2019].

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eHealth: Technology & Innovation

- Mobile devices & wearables
- Mobile health platforms
- Internet of Diagnostic Things (IoT)
- Biosensors
- Artificial Intelligence (AI)
- Digital Biomarker
- Other tools; i.e.,
  - QR barcodes,
  - Chips, Portable Battery,
  - Online data transfer

2559 2580 Medical Hub in Asian

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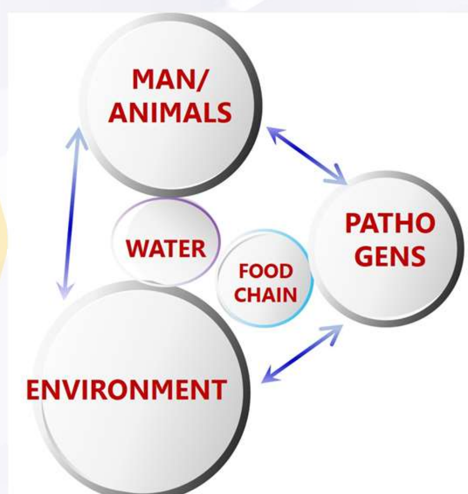
Application of Diagnostic device

- Early diagnosis
- Differential diagnosis
- Multi-disease diagnosis
- Multiplex testing
- Companion diagnostic (CDx)
- Point-of-care
- LIMCs
- Early warning system (EWS)
- Epidemiological study
- Identification pathogen
- Taxonomy



2559 2580 Medical Hub in Asian

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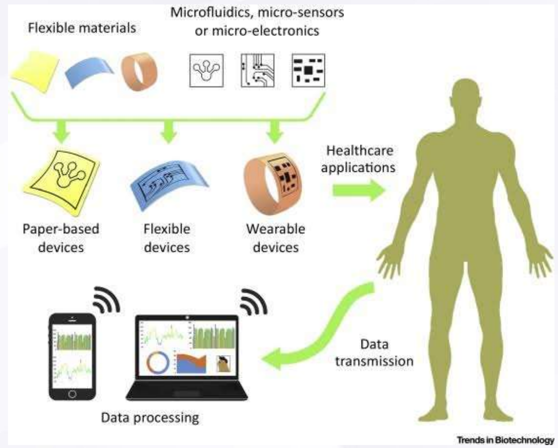
Trends of POC devices technology in clinical applications

- Flexible substrate
- Wearable devices
- Cloud-based POC diagnostics



Citation: Wang S, Chinnasamy T, Lifson MA, Inci F, Demirci U. Flexible substrate-based devices for point-of-care diagnostics. Trends Biotechnol. 2016; 34(11): 909-921.

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Outline:

4. Emergence of antimicrobial resistant (AMR) pathogens & control

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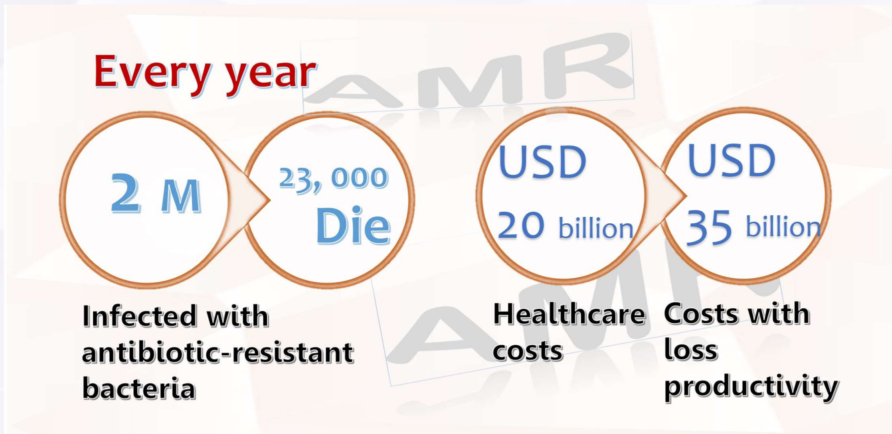
Multidrug Resistance (MDR) is an Emerging Threat

- Antimicrobial resistance (AMR) is present worldwide
- CDC estimates that:



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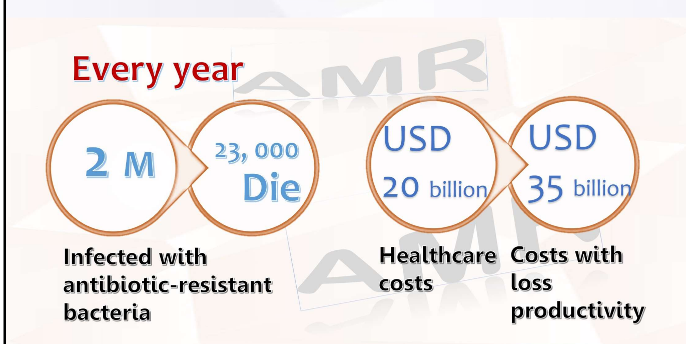
Emergence of drug-resistant pathogens



- Treatment failure
- Increased mortality and treatment costs
- Reduced infection control efficiency
- Spread of resistant pathogens from hospital to community.

Citation: Hashempour-Baltork F, Hosseini H, Shojaee-Aliabadi S, Torbati M, Alizadeh AM, Alizadeh M. Drug resistance and the prevention strategies in food borne bacteria: an update review. Adv Pharm Bull. 2019, 9(3): 335-347. doi: 10.15171/apb.2019.041

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The ESKAPE pathogens can effectively "escape" the effects of antimicrobial therapeutics

E*nterococcus faecium*
S*taphylococcus aureus*
K*lebsiella sp.*
A*cinetobacter baumannii*
P*seudomonas aeruginosa*
E*nterobacter sp.*

Antibiotic Resistance: Evolution & Dissemination

Inherent resistance
Genetic mutation
Horizontal gene transfer
- Transformation
- Transduction
- Conjugation

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Action Plan for Combating Antibiotic Resistant Bacteria

One Health: International collaboration for antibiotic-resistance prevention, surveillance, control, and antibiotic research and development.

Innovation development:

- Diagnostic tests for identification and characterization of resistant bacteria
- Novel antibiotics, other therapeutics, and vaccines

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A global AMR Surveillance system

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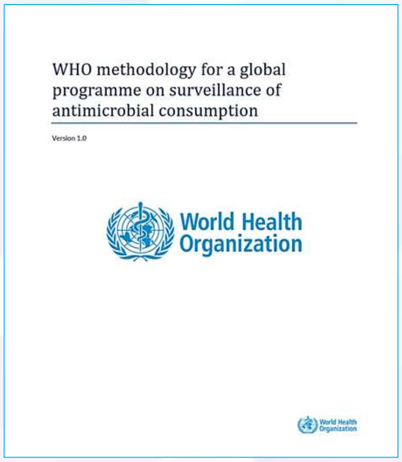
WHO methodology for a global programme on surveillance of antimicrobial consumption

Version 1.0

World Health Organization

WHO methodology for a global programme on surveillance of Antimicrobial consumption.

Citation: WHO methodology for a global programme on surveillance of antimicrobial consumption. Geneva: World Health Organization; 2015.

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A global AMR Surveillance system

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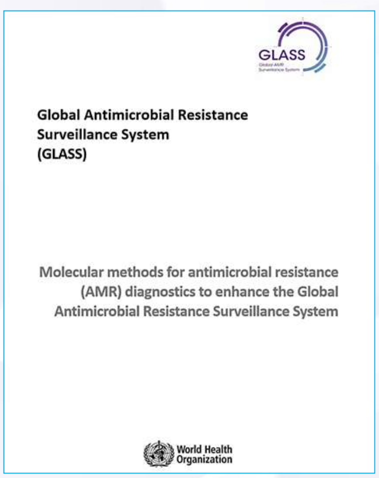
GLASS

Global Antimicrobial Resistance Surveillance System (GLASS)

Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System

World Health Organization

Global antimicrobial resistance surveillance system (GLASS)

Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System.

Citation: Molecular methods for antimicrobial resistance (AMR) diagnostics to enhance the Global Antimicrobial Resistance Surveillance System. Geneva: World Health Organization; 2019.

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Alternatives for traditional antibiotics to prevent or reduce the emergence of drug resistant bacteria.

MAN/ ANIMALS

- Farming practice
- Natural antibiotics
- Nano-antibiotics
- Lactic acid bacteria
- Bacteriocin
- Cyclopeptid
- Bacteriophage
- Synthetic biology and predatory bacteria

Citation: Hashempour-Baltork F, Hosseini H, Shojaee-Aliabadi S, Torbati M, Alizadeh AM, Alizadeh M. Drug resistance and the prevention strategies in food borne bacteria: an update review. Adv Pharm Bull. 2019, 9(3): 335-347. doi: 10.15171/apb.2019.041

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Outline:

5. Current development of therapeutic antibodies for FB and WB passive therapy

- Passive therapy using therapeutic antibodies
- Antibody engineering technology
- Passive therapy for leptospirosis
- Passive therapy for Influenza virus type A (FluA) infections

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Passive therapy using therapeutic antibodies

- Antibody is immunoglobulin (Ig), mostly found in serum
- widely used for therapeutic and diagnostic applications

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Passive therapy using therapeutic antibodies

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- 1890: *E. Behring* -> serum therapy using tetanus and diphtheria antitoxin serum prepared from immunized animals
- 1975: *G. Köhler and C. Milstein* invented monoclonal antibody technology
- Murine monoclonal antibodies are used for passive therapy of infectious & NCD, and intoxication.

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An ideal Therapeutic Antibody

- **Neutralizing/ Therapeutic activity (Broadly)**
  - Specificity to target
  - High binding affinity
  - Adequate recruitment of effector functions (Fc)
  - Non-immunogenic or minimized immunogenicity to patient
  - Desired half-life in the body
  - High target retention

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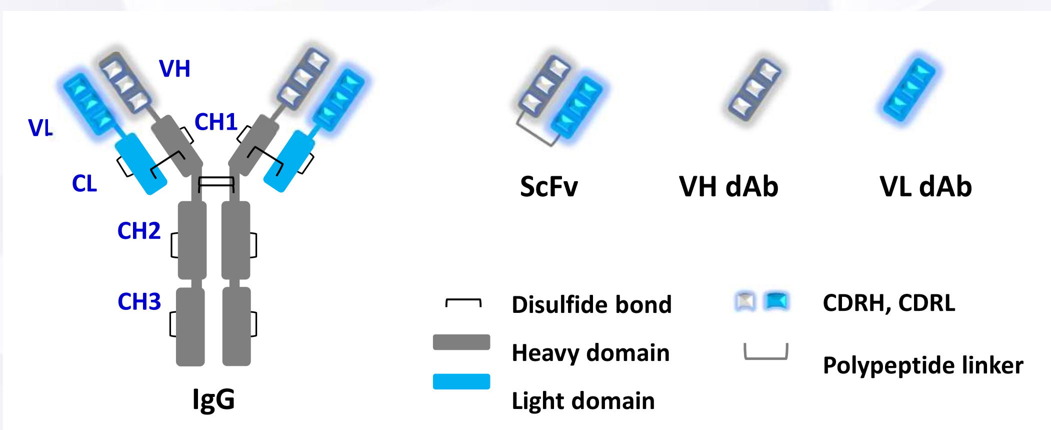
Antibody Molecule (IgG)



Citation: Maneewatchararangsri S. Therapeutic monoclonal antibodies and their engineered antibody fragments specific to LipL32 for passive immunotherapy of leptospirosis. *J Emerg Dis Virol.* 2016;2(2).

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Engineered Antibody Molecule

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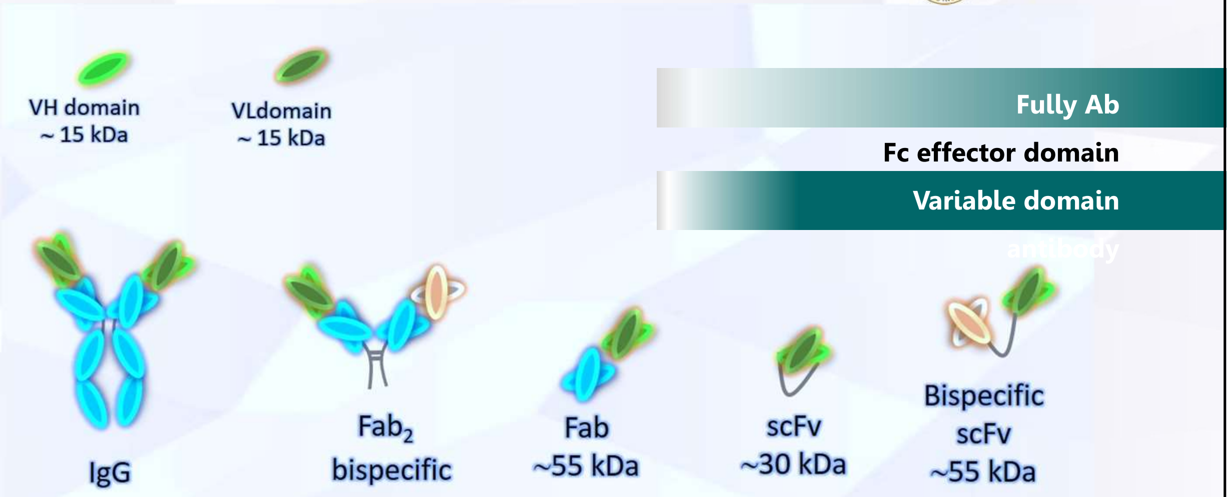

Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Fully Antibodies: cut and paste

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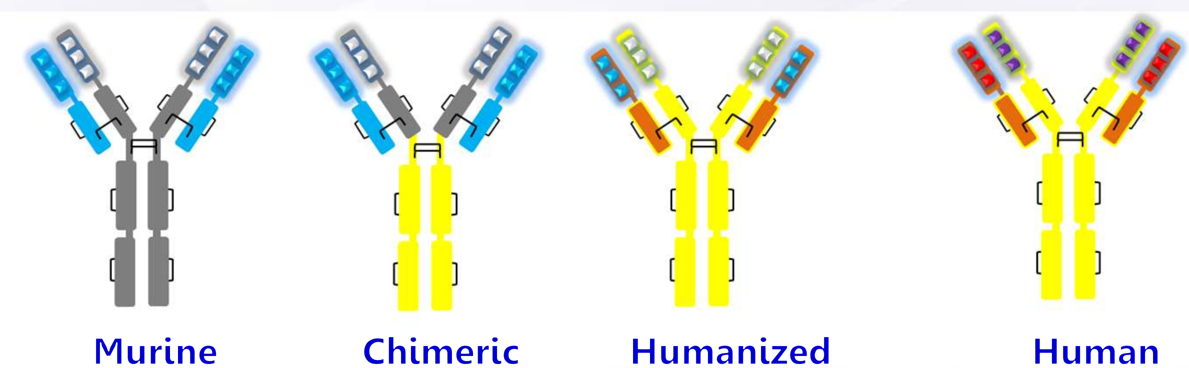

Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Antibody Molecule

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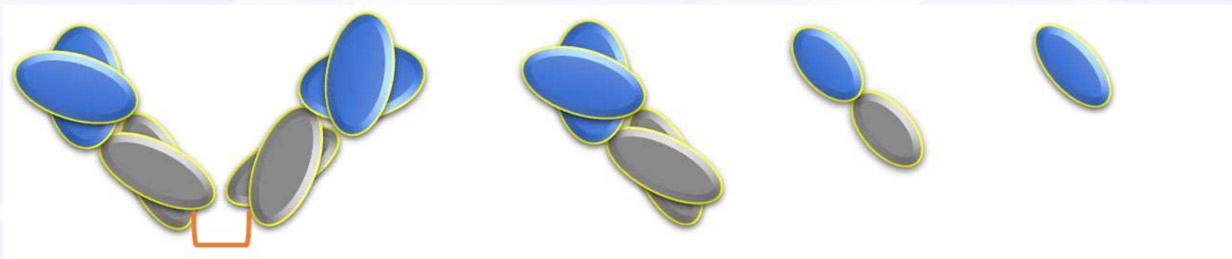
- Variable domain antibody



F(ab)′$_2$ Fab Fd [VH+CH1] VH/VL

Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Antibody Molecule

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- Variable domain antibody

scFv [VH-linker-VL] dimer- trimer- tetramer scFv

Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Antibody Molecule

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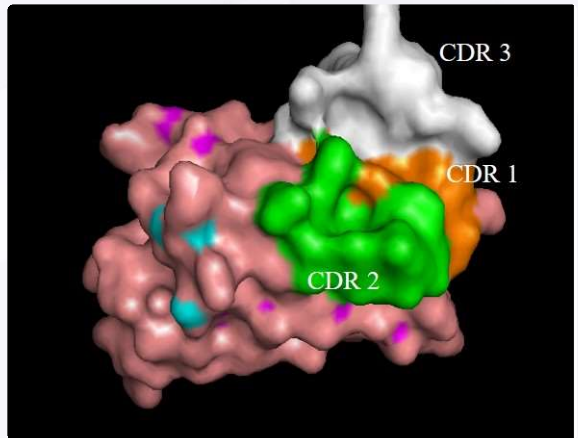
- **Hypervariable domain**

Variable domain of Heavy (VH) and VL chains contain hypervariable regions (CDR1, CDR2, and CDR3) responsible for binding specificity.



Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Antibody Molecule

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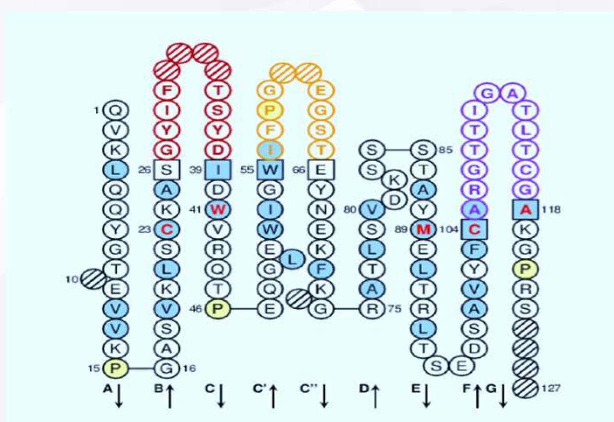
- Loops of hypervariable regions (CDR1, CDR2, and CDR3) responsible for binding specificity.

CDR1 CDR2 CDR3



Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Engineered Antibody Molecule

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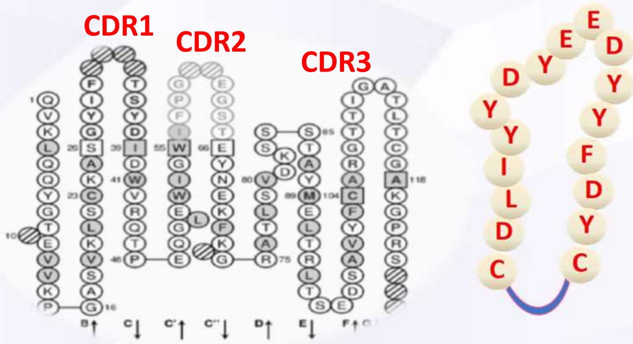
Cyclizied CDR loop



17- amino acids cyclized peptide derived from H-CDR3 of neutralizing Ab to HIV-1

(Millar *et al.*, 1998)

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Outline:

3. Current development of therapeutic antibodies for FB and WB passive therapy

- Passive therapy for leptospirosis
- Passive therapy for Influenza virus type A (FluA) infections

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Leptosprosis: Waterborne disease

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Zoonosis

Occupational disease

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Leptospirosis: Waterborne disease

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Water sport

Flooding

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Leptospirosis: Clinical manifestations

Acute undifferentiated febrile illness (AUFI)
Leptospirosis
Rickettsia; i.e. Scrub Typhus
Virus infection: Dengue, Flu
Bacterial septicemia: Melioidosis
Malaria
Undifferentiated

Severe leptospirosis
Weil's disease
Acute kidney failure
Pulmonary hemorrhage
Meningitis/ Meningoencephalitis
Myocarditis
Jaundice

Asymtomatic

Flu-like illness

Co-infections

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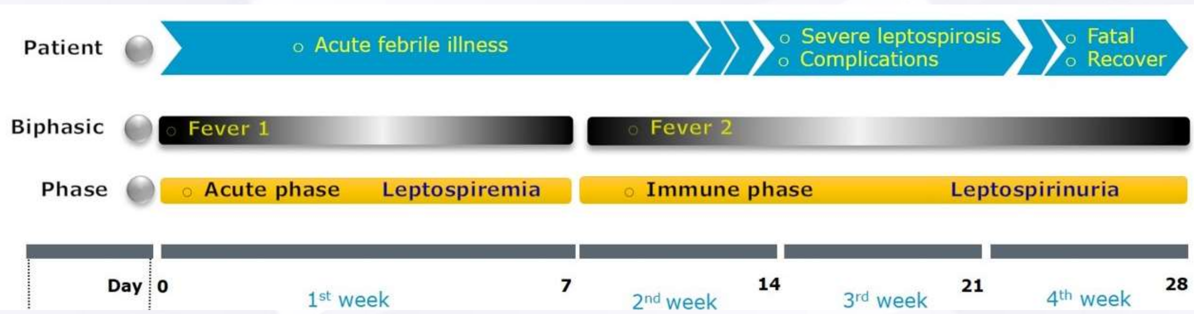
Leptospirosis: Clinical manifestations



Citation: Maneewatchararangsri S. Instructor. Personal Communication. 14 February 2020.

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Severe Leptospirosis patients

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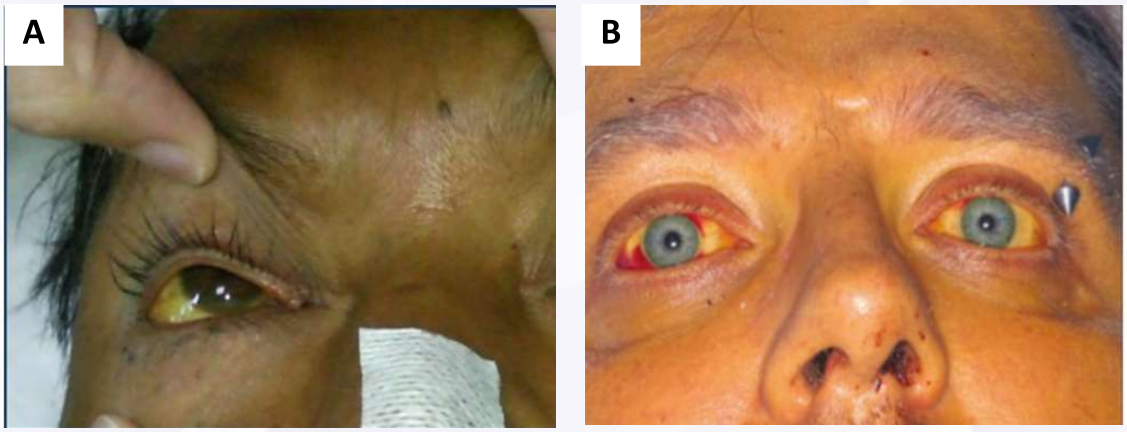
An icteric | **Hemorrhage**



A: Luveera V. Email send to: Santi Maneewatchararangsri. 31 January 2018.
B: Haake DA, Levett PN. Leptospirosis in humans. In: Adler B. (ed.) *Leptospira and Leptospirosis*. Springer-Verlag Berlin Heidelberg; 2015. p. 76.

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Leptospirosis: Therapy & Management

- Antibiotics: Doxycycline, Ceftriaxone-→ Ertapenem
- Anti-inflammatory drugs
- Best supportive ICU: Central line, Inotropic drugs, Ventilator support
- Hemodialysis
- Immunotherapy: alternative/ adjunct therapy

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Leptospirosis Passive Therapy using anti-LipL32 mAb and anti-LipL32 single chain variable fragment (ScFv)

Citations

Maneewatch S, Sakolvaree Y, Saengjaruk P, Srimanote P, Tapchaisri P, Tongtawe P, *et al.* Monoclonal antibodies to LipL32 protect against heterologous *Leptospira* spp. challenge. *Hybridoma* (Larchmt) 2008; 27(6): 453-65.

Maneewatch S, Sakolvaree Y, Tapchaisri P, Saengjaruk P, Songserm T, Wongratanacheewin S. Humanized-monoclonal antibody against heterologous *Leptospira* infection. *Protein Eng Des Sel.* 2009; 22(5): 305-312.

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Immunotherapy for leptospirosis: Therapeutic antibody and -fragments

Monoclonal Antibody (Mabs) to LPS

mAb to LipL32

mAb to LPS

Murine ScFv to LipL32
Humanized ScFv to LipL32

Adler and Faine, 1978; Jost *et al.*, 1986; Schoone *et al.*, 1989

Maneewatch *et al.*, 2008

Maneewatch *et al.*, 2008; 2009

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Histological appearances of Kidney sections

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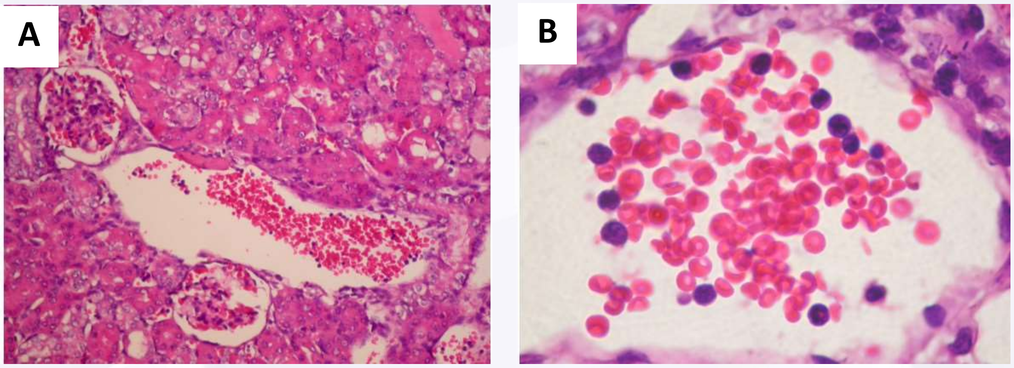
- **Humanized ScFv to LipL32 treatment**
  Interstitial nephritis (A; 200x) with mononuclear cells infiltrated in interstitial blood vessel (B; 1000x) were observed.



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Histological appearances of Kidney sections

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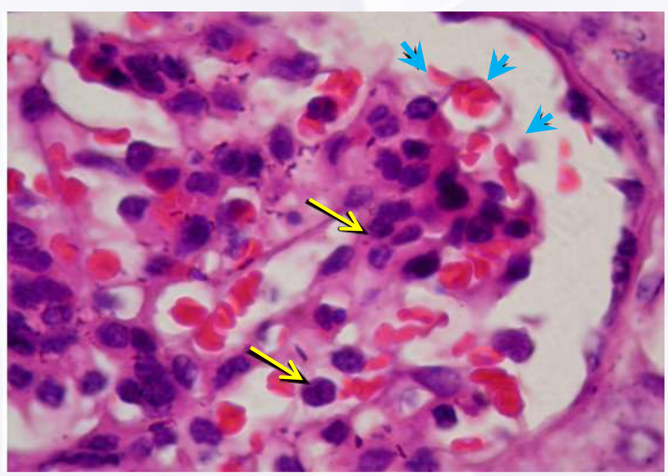
- **Humanized ScFv to LipL32**

Glomerulonephritis with a few mononuclear cells (arrows) and glomerular basement membrane degenerated (arrow head) were observed (1000x).



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Histological appearances of Kidney sections

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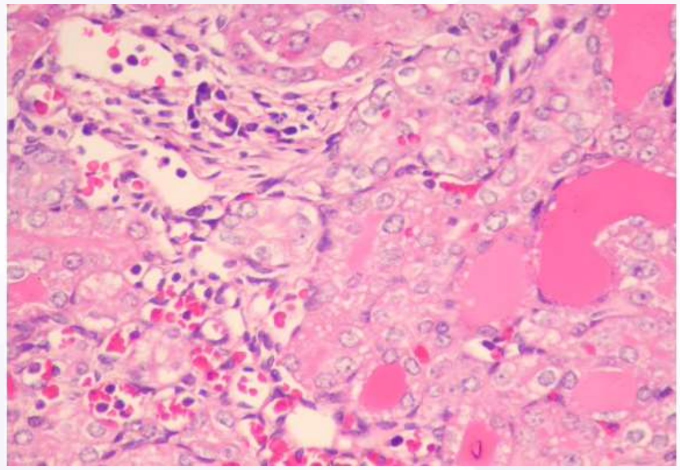
- **Humanized ScFv to LipL32**

Chronic interstitial nephritis with slight fibrosis was observed. Mononuclear cells infiltrated in fibrous tissue and interstitial blood vessels were found (400x).



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Histological appearances of Lung sections

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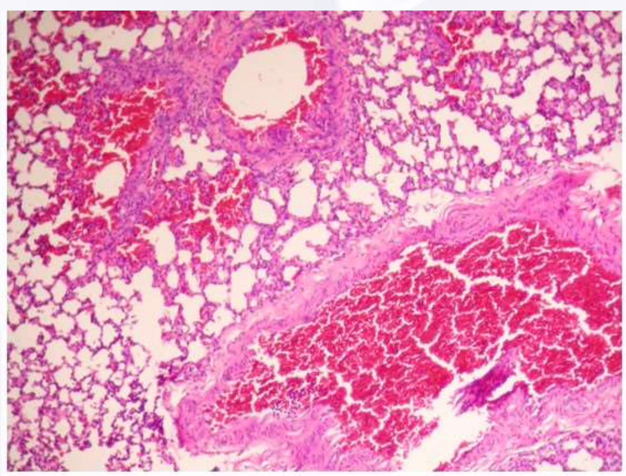
- **Humanized ScFv to LipL32**

Acute inflammatory cells infiltrated in blood vessel and aveolar septa (100x).



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Histological appearances of Lung sections

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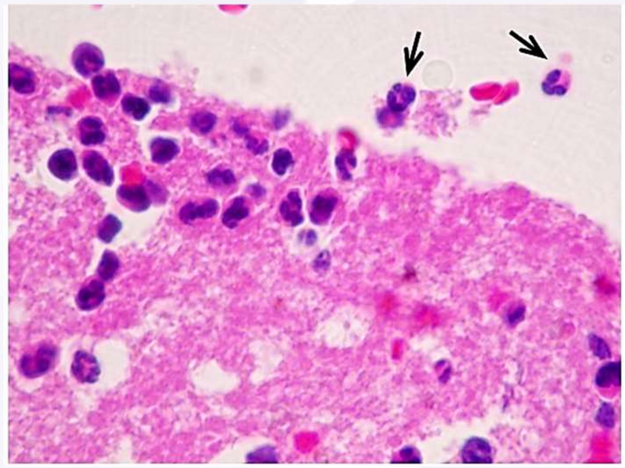
- **Humanized ScFv to LipL32**

Numerous neutrophils (arrows) were found in blood vessel with red blood cells hemolysis (400x).



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Histological appearances of Lung sections

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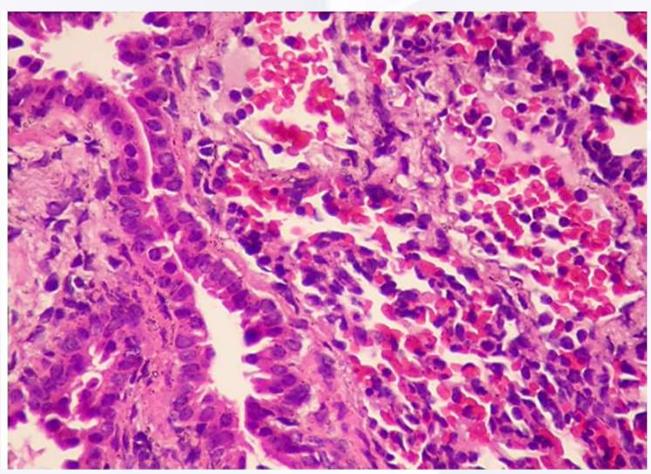
- **Humanized ScFv to LipL32**

mononuclear cells infiltration in blood vessels and alveolar septa (400x).



Adapt from Maneewatchararangsri, S (2007).

Citation: Maneewatchararangsri, S (2007). Humanized-monoclonal antibody that neutralizes heterologous *Leptospira* infection. (Doctoral dissertation) Thammasat University Thailand.

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Passive Therapy of Influenza A subtype H5N1 infection using human single chain antibodies to hemagglutinin

Citation:

Maneewatch S, Thanongsaksrikul J, Songserm T, Thueng-In K, Kulkeaw K, Thathaisong U, et al. Human single-chain antibodies that neutralize homologous and heterologous strains and clades of influenza A virus subtype H5N1. Antivir Ther. 2009; 14(2): 221-30.

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Pandemic/epidemic preparedness

1. Vaccine development: Broad spectrum vaccine based on conserved influenza virus proteins
2. Invention of new family of anti-virus drugs
3. Invention of therapeutic antibodies (alternative)

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Treatment of influenza with current antiviral drugs

- **M2 ion channel blockers:** Amantadine, Rimantadine >> RESISTANCE
  (Li *et al.*, 2004; Puthavathana *et al.*, 2005)
- **Neuraminidase inhibitors:** Oseltamivir, Zanamivir>> RESISTANCE
  (Le *et al.*, 2005; de Jong *et al.*, 2005)

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Obstacles of Anti-influenza drugs

- Drugs resistant mutants: antigenic drift & shift
- Must be given early in the course of illness
- High demand, limited supply during the epidemic / pandemic
- New drug family ?????

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Antibodies therapy for Influenza A infection

- Transfusion of **human blood products** from patients recovering from "Spanish flu" can reduced 50% mortality of influenza.
- **Specific MAbs to influenza A** confer prophylactic and therapeutic protection in mice.
- **Humanized and equine F(ab')$_2$ fragments** specific to H5N1 have used for prophylaxis and therapy in the mouse model.

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Antibodies therapy for Influenza A infection

- Antibodies may be used in the late stage on infection when the anti-viral drugs fail to reduce the systemic viral load and ameliorate the severity of the symptoms (Zhou *et al.*, 2007)
- Human single chain antibody (HuScFv) specific to HA of H5N1 virus neutralize various strains and clades of H5N1 influenza A viruses (Maneewatch *et al.*, 2009)

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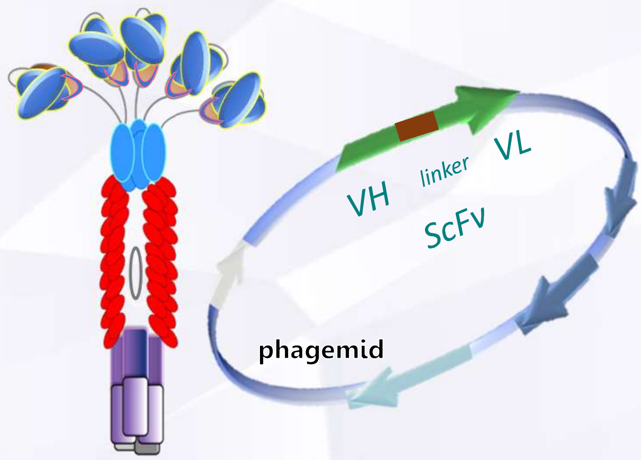
Structure of ScFv-displaying filamentous phage



Human scFv phage display antibody library

2.6 x 10^8 HuScFv phage display single pot library

(Kulkeaw *et al.*, 2009)

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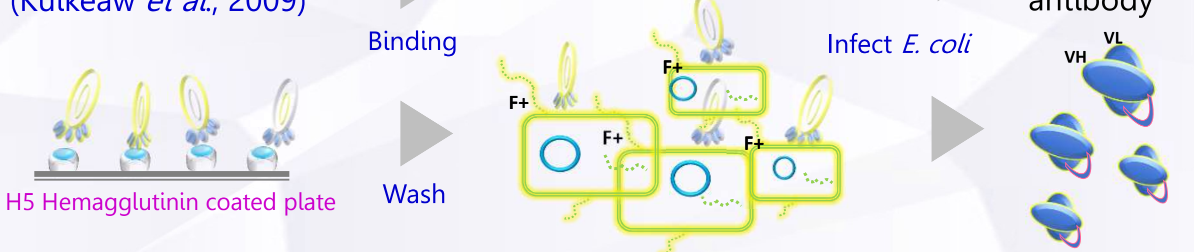
Phage bio-panning for selecting phage clones displaying human ScFv (HuScFv) specific to recombinant HA

2.6 x 10^8 Human scFv phage display single pot library (Kulkeaw *et al.*, 2009)

Human scFv phages specific to H5 Hemagglutinin

Soluble Human scFv antibody



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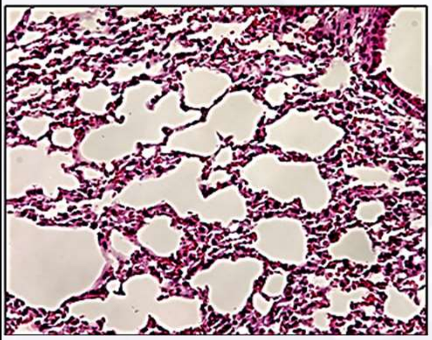
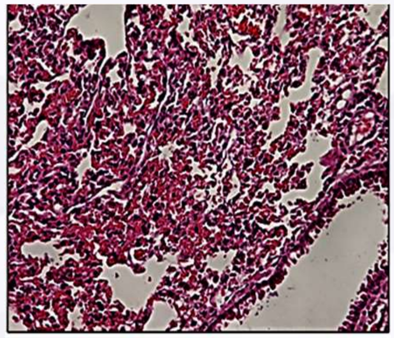
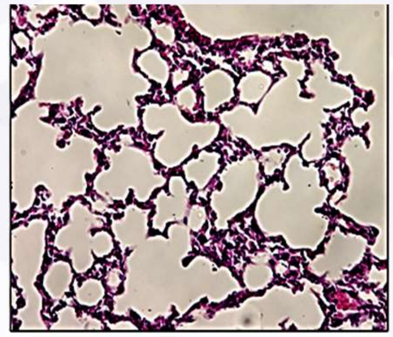
HuScFv treatment could reduce lung histopathology



Human ScFv treated, 5 mg/kg/dose



Infected lung tissue



Normal lung tissue

20 February 2020

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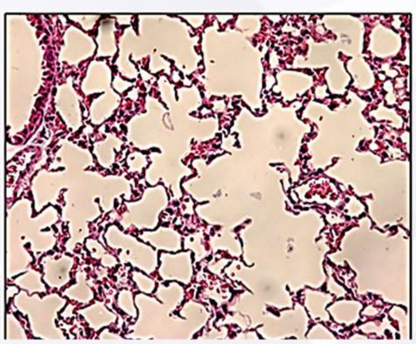
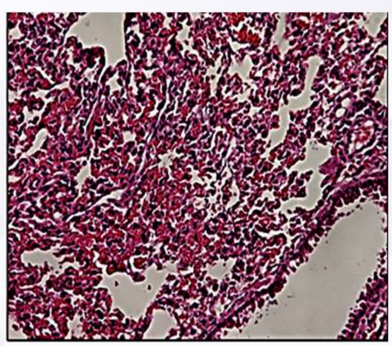
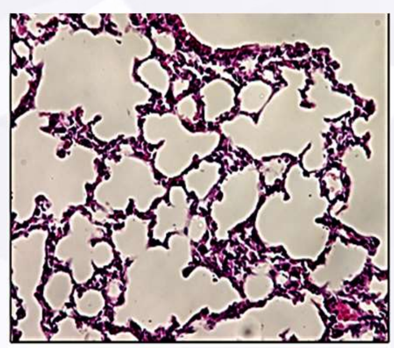


Human ScFv treated, 10 mg/kg/dose



Infected lung tissue



Normal lung tissue

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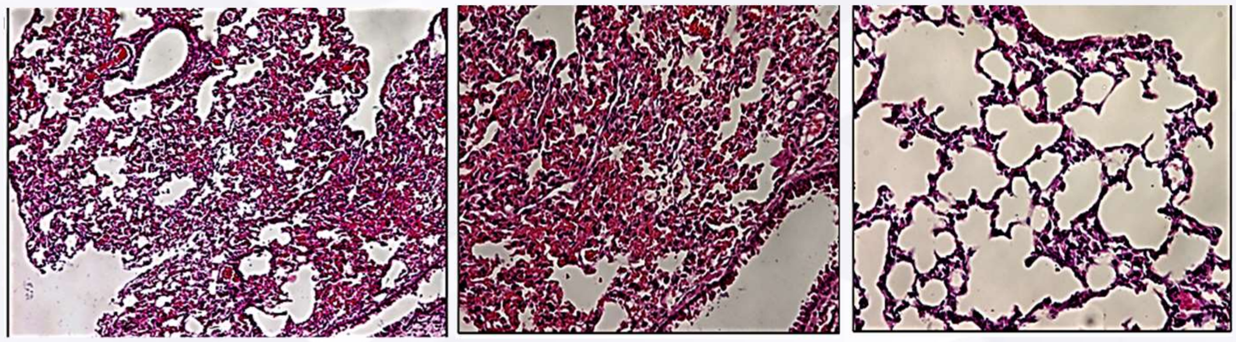

Irrelevant
Human ScFv treated

Infected lung tissue

Normal lung tissue

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THAILAND 4.0
Prosperity, Security, Sustainability

END OF SESSION

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