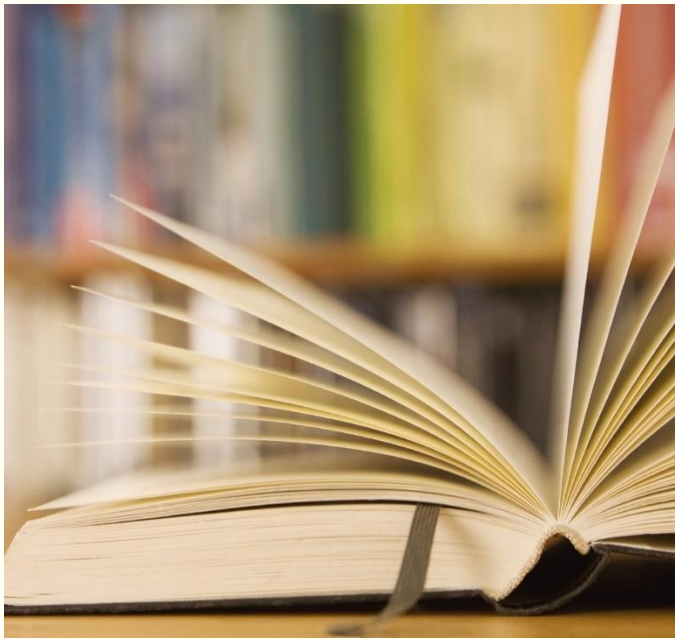
BIOSAFETY REGULATION AND GUIDELINES FOR INFECTIOUS MICROORGANISMS



Potjanee Srimanote
Chair, Thammasat University Biosafety Committees
January 29, 2021

Translated from “Law and regulation on biosafety and biosecurity” May 21, 2019

National Center for Genetic Engineering and Biotechnology (BIOTEC), Policy Study and Biosafety Unit

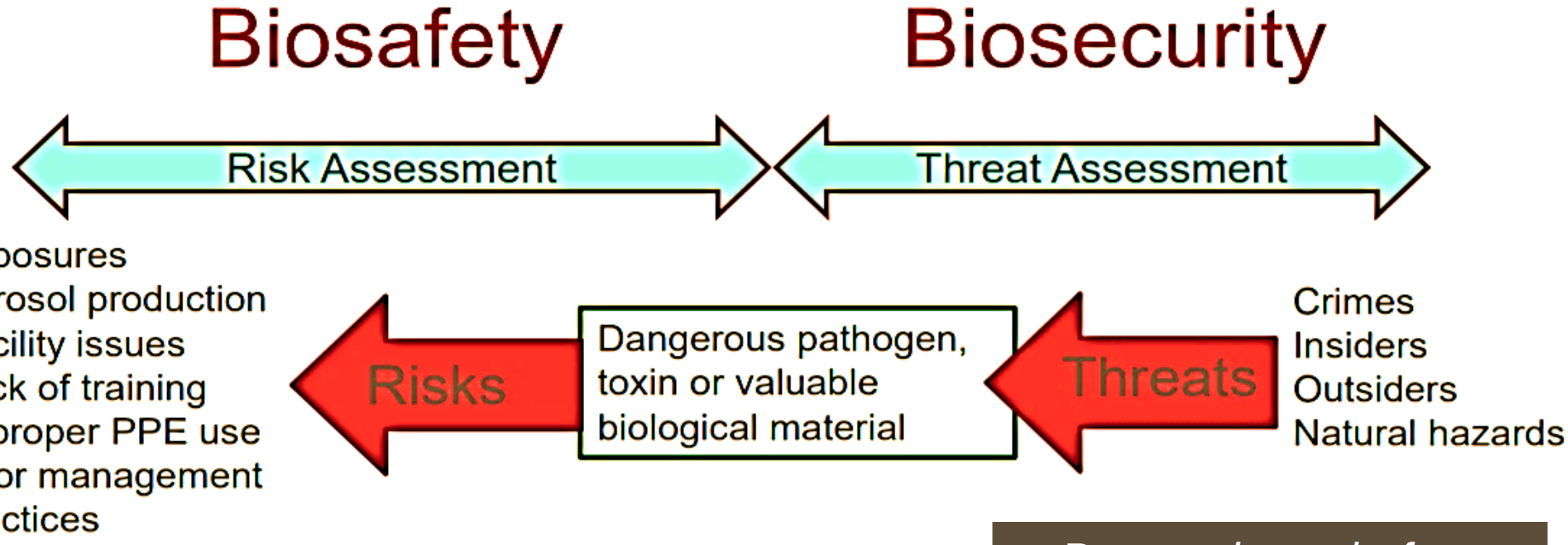
Objective and scope

To provide knowledge on the requirement and regulation involving in biosafety and biosecurity in Thailand.

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

BIOSAFETY GUIDELINES for Modern Biotechnology, BIOTEC, 2016

Laboratory Biosafety vs Biosecurity
in bioscience or biomedical research (2)



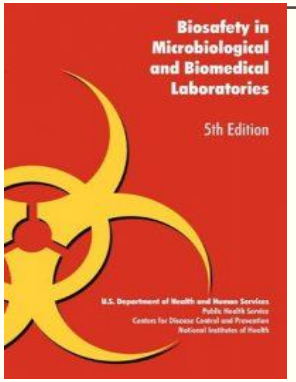
- *Reduce* or eliminate *exposure*
- **Accidental release**

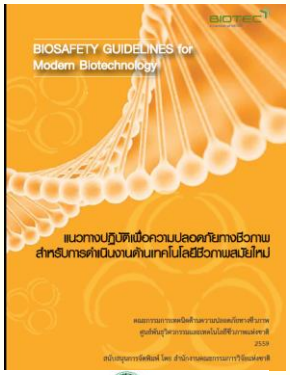
- *Prevent loss, theft*
- **Intentional release**
- *Limit access*

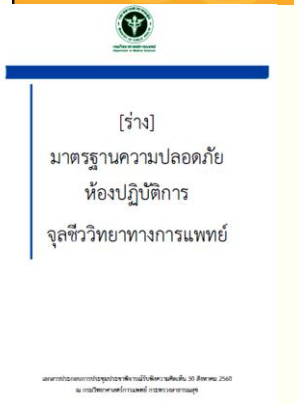
Important of Biosafety

- Laboratory workers recognize hazards of processing infectious agents
- Laboratory biosafety aims to reduce or eliminate exposure of laboratory workers, or other persons, and the outside environment to potentially infectious agents involved in bioscience research.
- Laboratory biosafety is **achieved** by implementing various degrees of laboratory **“containment”, or safe methods** of managing infectious agents.
  - Through engineering controls, management policies and work practices
  - Labs must adhere to very specific safety regulations to work with organisms that pose a threat to human health

Recommended National biosafety guidelines for research use of microbial agents/pathogens and toxins.







1. Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Eds

Knowledge sufficient to work safely with hazardous microorganisms and the biosafety principles

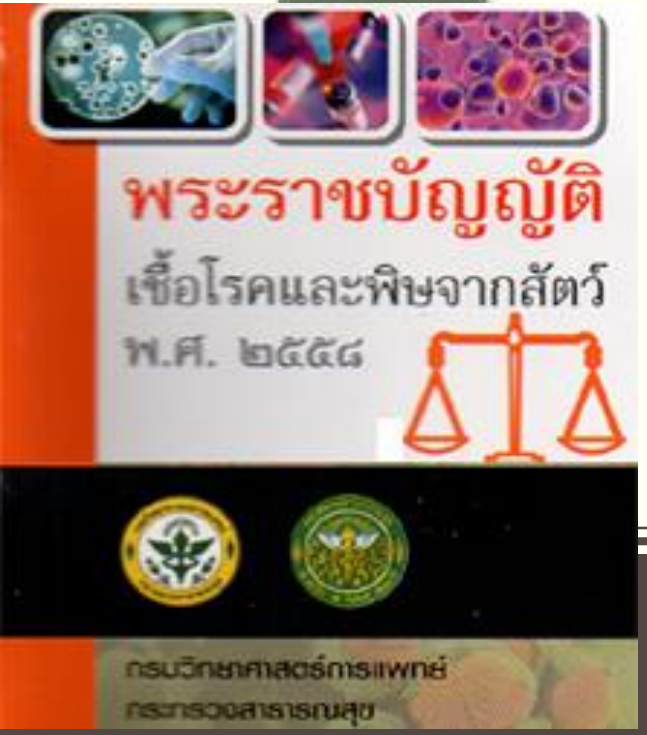
https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF

2. BIOSAFETY GUIDELINES for Modern Biotechnology.

http://www.biotec.or.th/biosafety/images/document/G01-Biosafety%20Guideline.pdf

3. (Draft) คู่มือมาตรฐานความปลอดภัยห้องปฏิบัติการจุลชีวิทยาทางการแพทย์

http://nih.dmsc.moph.go.th/login/filedata/1.pdf



PATHOGENS AND ANIMAL TOXINS ACT,

B.E. 2558 (2015)

BHUMIBOL ADULYADEJ, REX.

Given on the 20th Day of August B.E. 2558;

Being the 70th Year of the Present Reign.

NATIONAL LAWS AND REGULATION

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

http://blqs.dmsc.moph.go.th/assets/Bpat/ACTforBPAT.pdf (English)

http://niah.dld.go.th/th/files/legislation/act_microbe_toxin_2558_2bx.pdf (Thai)

http://blqs.dmsc.moph.go.th/page-view/99 (Bureau of Laboratory Quality Standards)

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

Department of Medical Sciences, Ministry of Public Health)

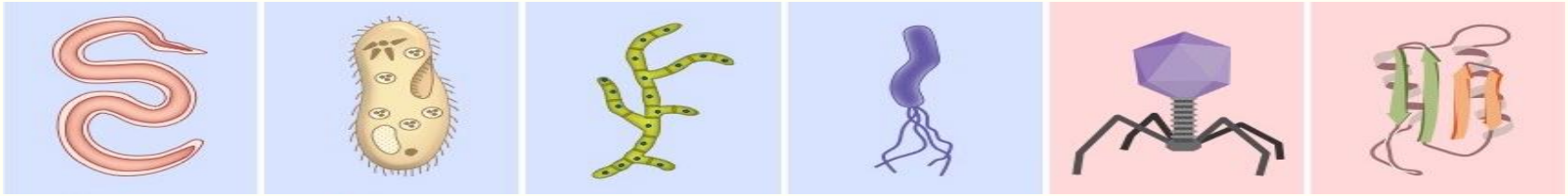
Objective:
To maintain the safety and welfare of public from the pathogens and animal toxins

- Regulation is covered on the operation on **pathogens and animal toxins** that effect to humans, animals, livestock and beasts of burden (animals that are used as vehicles)
- Pathogens that are **causing diseases exclusively in plants** are excluded from the Act

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)
Definition (Section 4. In this Act):1

"**Pathogen**" means: (1) Microorganisms;
(2) Biological substance (สารชีวภาพ);
(3) other germs listed in the Notifications issued under section 6 (3).

(1) Microorganisms = Parasites, protozoa, molds/yeast, bacteria, and viruses



2) **Biological substance** *means:*

2.1) a culture/ any part/product of microorganisms or animal toxin e. g. DNA, RNA , their toxin and protein

2.2) Proteinaceous infectious particle (**Prion**)

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)
Definition (Section 4. In this Act): 2

- **"Animal toxin"** means

Toxin from animals that **causes a condition that prevents the body from working normally** in humans, livestock, beasts of burden or other animals prescribed in the Notifications issued under section 6 (3)

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

The scope : regulated activities:

Posess

- **Possess =** To have in possession for the possessor himself or herself or for other person

Produce

- **Produce** = To cultivate, mix, aliquot, transform, add volume, synthesize, repacking and infect animals

Sell

- **Sell** = To dispose of, distribute, dispense or **exchange** for trade

- **Import , export, transit**

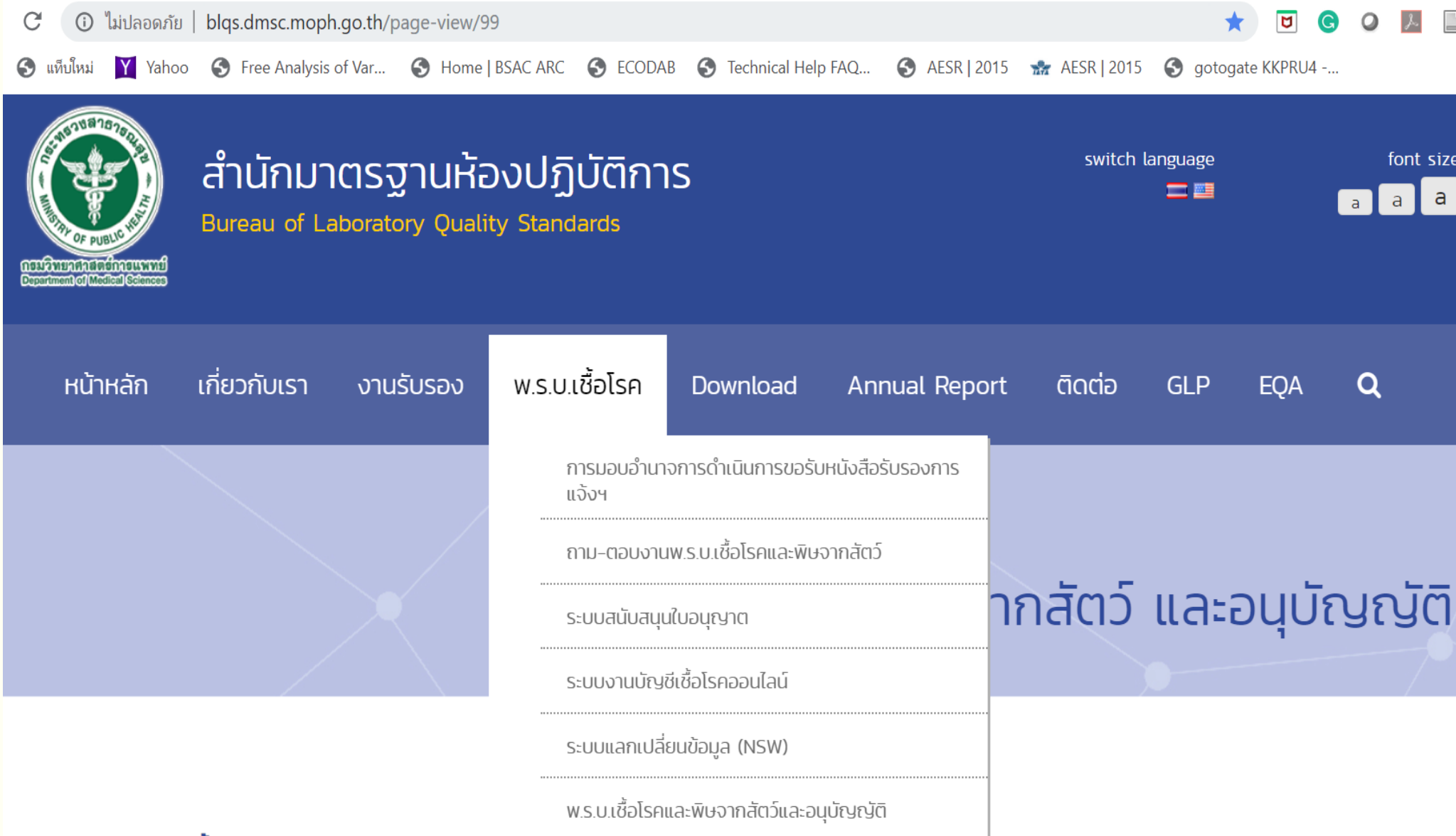
- Any Institute/unit who intends to **carry out these activity for Risk Groups 1 and 2 Pathogens** under section 18 or **Group 1 Animal Toxins** under section 19 shall notify the Director-General in writing in order to obtain a ***certificate of notification*** (Section 20 and 21).
- **Carry out these activity for Risk Groups 3 Pathogens** or **Group 2 Animal Toxins** shall file an *application for a* ***license*** with the Director-General (Section 22).

Medical and Animal Laboratories

พรบ.เชื้อโรคฯ | **Biosafety and Biosecurity**

1. **Research/Commercial Laboratory**
   - **1.1 Pathogen/animal toxin**
   - **1.2 Non-pathogen/animal toxin**
   - **1.3 Analytical Lab** (ยา อาหาร ผลิตภัณฑ์สุขภาพ สิ่งแวดล้อม)
2. **Clinical Laboratory (Diagnostics)**
   - **2.1 Microbiology (ATCC reference strain)**
   - **2.2 Non-Microbiology**
   - **2.4 Autopsy**

ให้ทำลายหรือส่งมอบ แต่ไม่ใช้บังคับกรณีครอบครองเพื่อการตรวจวินิจฉัยและชันสูตร/ มาตรา 6 (7) (8)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Department of Medical Sciences
สำนักมาตรฐานห้องปฏิบัติการ
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การมอบอำนาจการดำเนินการขอรับหนังสือรับรองการแจ้งฯ
ถาม-ตอบงานพ.ร.บ.เชื้อโรคและพิษจากสัตว์
ระบบสนับสนุนใบอนุญาต
ระบบงานบัญชีเชื้อโรคออนไลน์
ระบบแลกเปลี่ยนข้อมูล (NSW)
พ.ร.บ.เชื้อโรคและพิษจากสัตว์และอนุบัญญัติ
รายชื่อหน่วยงานที่ได้รับใบอนุญาตและหน่วยงานที่ได้รับหนังสือรับรองการแจ้ง
ากสัตว์ และอนุบัญญัติ
- พระราชบัญญัติเชื้อโรคและพิษจากสัตว์ พ.ศ. ๒๕
- PATHOGENS AND ANIMAL TOXINS ACT, B.E

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

- For the **purpose of pathogens control**, the Notified pathogen list are classified into 4 groups (Section 18)

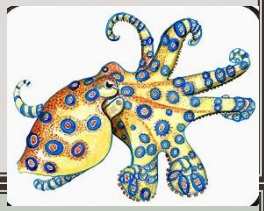
  - Based on **prevention** and **treatment** methods, spreading and **quantities or amount of pathogens**, and the **degree of risk of causing diseases** to humans, the community, livestock, beasts of burden or other animals.

Group 1 (RG1) Low risk	**Rarely cause infection** in healthy hosts. Examples: specific laboratory strains *E. coli*, *S. cerevisiae, P. pastoris* and some lactobacilli
Group 2 (RG2) Moderate risk	Agents may **cause disease** in a healthy host but are **difficult to transmit**, **do not** usually **cause serious or life-threatening illness** and are **readily treated or prevented**. Examples: pathogenic *E. coli*, *Shigella* spp, *Plasmodium spp*
Group 3 (RG3) High risk	Agents usually **cause serious disease/** serious risk to laboratory workers/ **present significant community risk if spread in the environment**, but there are usually **effective measures for treatment and/or prevention**. Examples: *B. anthracis*, hantavirus, yellow fever, HIV (cultures), SARS-CoV-2
Group 4 (RG4) Very high risk	Agents are those that present **significant individual and community risks and usually produce life-threatening disease**, are readily transmissible and effective **prevention and/or treatment is not usually available**. Examples: Ebola, Hendra and Nipah viruses.

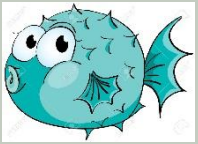
For the **purpose of Animal toxin control**, the Notified pathogen list are classified into 3 groups **(Section 19))**

Based on **treatment methods and quantities or amount** of animal toxins, in consistence with the **degree of risk of causing a condition that prevents the body from working normally** which may occur in humans, livestock, beasts of burden or other animals

Group 1 Animal Toxins → cause a condition that **prevents the body from working normally, but not at a serious level** and effective **treatment is available**;



Group 2 Animal Toxins →cause a condition that **prevents the body from working normally at a serious level** and effective **treatment is available**



Group 3 Animal Toxins → cause a condition that prevents the body from working normally at a serious level and effective **treatment is not available**

http://blqs.dmsc.moph.go.th/assets/Bpat/PATratchakitcha182561.pdf

เล่ม ๑๓๕ ตอนพิเศษ ๓๐๑ ง ราชกิจจานุเบกษา ๒๗ พฤศจิกายน ๒๕๖๑

ประกาศกระทรวงสาธารณสุข

เรื่อง รายการเชื้อโรคที่ประสงค์ควบคุมตามมาตรา ๑๘

พ.ศ. ๒๕๖๑

Notification of the Ministry of Health

Entitle "the list of pathogens intended to be under the control under section 18"

B.E 2561 (2018)

(๑) ตัวอักษรภาษาอังกฤษที่ใช้แทนประเภทของเชื้อโรค ได้แก่

B ใช้แทนแบคทีเรีย

F ใช้แทนรา

V ใช้แทนไวรัส

P ใช้แทนปรสิต

T ใช้แทนสารชีวภาพที่ไม่ใช่อนุภาคโปรตีน

R ใช้แทนอนุภาคโปรตีนก่อโรค

→ ***Abbreviation***

B = bacteria

F = fungi, mold yeast

V = virus

P = parasite (helminth, protozoa)

T = toxin/ Non-Proteinaceous infectious particle biological substance

R = Proteinaceous infectious particle (**Prion**)

Code	Scientific name of controlled pathogen	Group (RG)	Affected host	Additional information

รหัสเชื้อโรค	ชื่อเชื้อโรคควบคุม	กลุ่มที่	การก่อโรค	รายละเอียดเพิ่มเติม
Bacteria				
B-2-0300	*Brevundimonas* spp.	๒	คนและสัตว์	หมายความว่า เป็นเชื้อที่ไม่สามารถ วินิจฉัย species ได้
B-1-0301	*Brochothrix thermosphacta*	๑	คนและสัตว์	
B-1-0302	*Brochothrix* spp.	๑	คนและสัตว์	หมายความว่า เป็นเชื้อที่ไม่สามารถ วินิจฉัย species ได้
B-3-0303	*Brucella abortus*	๓*	คนและสัตว์	

2

Human + animal

1

1

3*

3* is the RG3 agents that the Notification allows some of the activities (without cultivate) to **carried out in BSL-2-enhance**

BSL-2-enhance = BSL-2 facility (isolated room + BSL-3 –type personal protective equipment

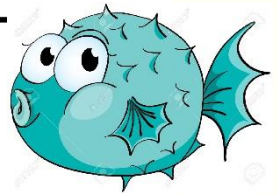
Code	Scientific name of controlled pathogen	Group (RG)	Affected host	Additional information
รหัสเชื้อโรค	ชื่อเชื้อโรคควบคุม	กลุ่มที่	การก่อโรค	รายละเอียดเพิ่มเติม
T-3-0065	T-2 Triol	๓*	คน	
T-2-0066	Tenuazonic acid	๒	คน	
T-3-0067	Tetanus Toxin	๓*	คนและสัตว์	
T-3-0068	Tetrodotoxin	๓*	คน	
T-3-0069	Trichothecenes	๓*	คน	
T-2-0070	Verruculogen	๒	คน	
T-2-0071	Vomitoxin	๒	คน	
T-3-0072	Zearalenone	๓*	คนและสัตว์	
อนุภาคโปรตีนก่อโรค				
R-3-0001	Bovine spongiform encephalopathy prion and other related animal transmissible spongiform encephalopathy prion	๓*	คนและสัตว์	
R-3-0002	Chronic Wasting Disease	๓*	คนและสัตว์	
R-3-0003	Creutzfeldt-Jakob disease prion	๓*	คน	

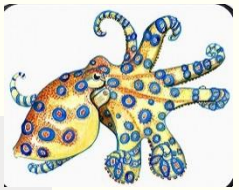
human

Human + animals

T* = *toxin/ Non-
Proteinaceous infectious particle biological substance

R = Proteinaceous infectious particle (**Prion**)





ประกาศกระทรวงสาธารณสุข

เรื่อง รายการพิษจากสัตว์ที่ประสงค์ควบคุมตามมาตรา ๑๙

พ.ศ. ๒๕๖๐

Notification of the Ministry of Health

Entitle "the list of Animal Toxin intended to be under the control under section 19"

B.E 2560 (2017)

Code	Scientific name of controlled pathogen	Risk group (human)	Risk group (animals)	Additional information

ลำดับที่	ชื่อพิษจากสัตว์ควบคุม	กลุ่มที่ (เสี่ยงต่อคน)	กลุ่มที่ (เสี่ยงต่อสัตว์)	เงื่อนไข
๑	*Acanthophis antracticus*	๓	๓	
๒	*Acanthophis praelongus*	๓	๓	
๓	*Acanthophis pyrrhus*	๓	๓	
๔	*Acnathaster planci*	๒	๒	
๕	*Acromitus rabanchatu*	๒	๒	

PATHOGENS AND ANIMAL TOXINS ACT, B.E. 2558 (2015)

Mechanism of control/Code of practice

Low risk	Moderate risk	High risk	Very high risk
Good microbiological practice (GP)	**Obtaining a certificate of notification**	***License application***	Prohibited
Pathogen RG1	Pathogen RG2 Animal toxin G1	Pathogen RG3 Animal toxin G2	Pathogen RG4 Animal toxin G3
Operate in BSL-1 to BSL-4	Operate in BSL-2 to BSL-4	Operate in BSL-3 to BSL-4	Operate in BSL-4

RESEARCH PROJECT CATEGORY FOR MICROORGANISMS & MODERN BIOTECHNOLOGY

Acknowledgements

"Biosafety Guidelines" course development team

Assoc. Prof. Prasartporn Samitaman
Prof. Srisin Khusmith
Dr. Chaisuree Suphavilai
Dr. Kloyjai Samretwanich
Dr. Boonhiang Promdonkoy
Dr. Chalinee Kongsawat
Ms. Namol Vorapreeda
Ms. Jintana Chancharoenrit
Ms. Siras Sulanchupakorn

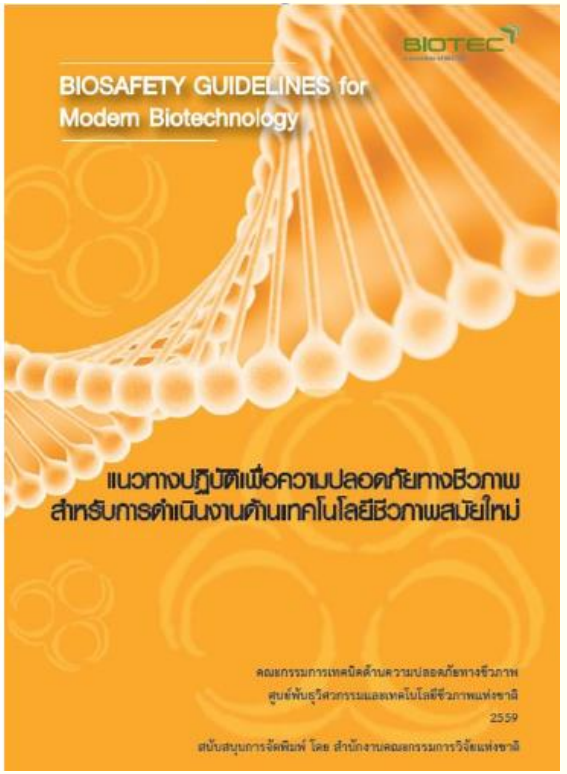
COMPONENTS INVOLVING IN GENE MODIFICATION

such as vector, plasmid, cosmid, virus, bacteria, promoter, enhancer, selectable marker etc.

PATHOGENS AND ANIMAL TOXINS ACT

Points to consider

- Source of components
- Pathogenicity
- Potential of gene transfer
- Selectable marker gene (if any) especially antibiotic resistant gene



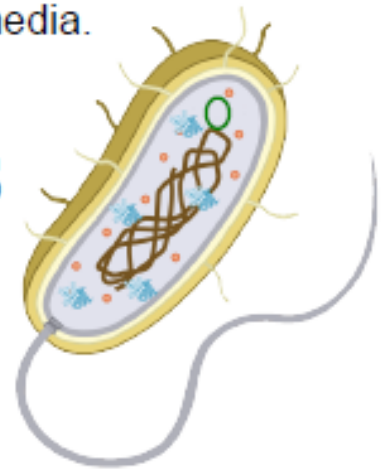
INSERTED DNA/ TARGET GENE

Points to consider

- Source of inserted DNA and target gene
- Characteristics of gene product
  - o Involves in carcinogenesis and immune system
  - o Affects the functioning of other gene(s)
  - o Toxin

Risk groupตาม พรบ. เชื้อโรค

GENETECALLY MODIFIED ORGANISMS



Points to consider

- Competitive advantage over the native organisms
- Availability of effective preventive and control methods
- Pathogenicity and toxicity
- Purpose of use: in containment or environmental release
- Effects to ecosystem

RISK CATEGORY IN BIOSAFETY GUIDELINE

ประเภทงานวิจัย C1-C4

Category 1	Category 2	Category 3	Category 4
Low risk hazard	Moderate risk hazard	High risk hazard/ involving the treatment of patients using r-DNA technology/ unverified risk	Very high/ unacceptable risk hazard or considered immoral in principle

Do not allow

CATEGORY 1 LOW RISK EXPERIMENT TO LABORATORY STAFF, COMMUNITY AND ENVIRONMENT

The following items are within the coverage of category 1

1. Those that consist entirely of DNA segments from different species that exchange DNA by known physiological processes (see appendix A-I through A-VI - NIH guideline, 2016).
2. Those that using safety-approved host and vector system (see appendix C - NIH guideline, 2016).
3. Those that generated by self-cloning in microorganisms/agents considered low risk hazard (with evidence). **Same species of RG1 pathogen**

The following items are within the coverage of category 1

4. Those that experiments in which the DNA from a particular host organism is propagated only in that same organism.
   - In case of plant: must not exhibit weedy characteristic or that must not be capable of interbreeding with weeds or related species growing in the vicinity.
   - In case of animal: must not be an invasive alien species.

Including other work on ***Risk group 1 pathogen*** from Pathogen and Animal Toxin Act

CATEGORY 2 MODERATE RISK HAZARD TO THE LABORATORY STAFF, COMMUNITY AND ENVIRONMENT

The following items are within the coverage of category 2

1. Those that using host and vector system that is not listed in safety-approved (see appendix C - NIH guideline, 2016).
2. Those that using safety-approved host and vector system (see appendix C - NIH guideline, 2016) with the following transgene:
   - Toxin gene สารชีวภาพ Risk group 2/ animal toxin group 1 จาก พรบ. เชื้อโรค
   - Human, animal and plant pathogen DNA of organisms classified in risk group 2 (see appendix B-II - NIH guideline, 2016).
   - Cellular growth-effecting protein genes; e.g. oncogenes
3. Those that using organisms classified in risk group 2 (see appendix B-II - NIH guideline, 2016). เชื้อโรค Risk group 2 จาก พรบ. เชื้อโรค

The following items are within the coverage of category 2

4. Those that generated by self-cloning in microorganisms/agents considered to be moderate risk (with evidence).
5. Transgenic plants/ animals that does not contain DNA from exotic infectious agents.

Clone ยีน ทำ mutant ในเชื้อโรค Risk group 2 จาก พรบ. เชื้อโรค
Clone ยีน จากเชื้อโรค Risk group 2 จาก พรบ. เชื้อโรค ไปยัง *E. coli* RG1

CATEGORY 3 HIGH RISK HAZARD TO THE LABORATORY STAFF, COMMUNITY AND ENVIRONMENT, OR INVOLVING THE TREATMENT OF PATIENTS USING r-DNA TECHNOLOGY OR PROJECTS WITH UNVERIFIED RISK

The following items are within the coverage of category 3

1. Those that using host and vector system, genes or DNA fragments from potential human, animal and plant pathogens classified in risk group 3 (see appendix B-III - NIH guideline, 2016) or unverified risks.

Including other work on ***Risk group 3 pathogen*** from Pathogen and Animal Toxin Act

The following items are within the coverage of category 3

2. Experiment involving toxin-producing organisms, DNA or DNA cloning of gene(s) producing toxin with LD50<100 ng/kg bodyweight (BW), high-yielding toxin with LD50>100 ng/kg BW; microbial DNA potentially containing toxin genes for unknown toxins. The proposal must describe the type of the toxin, organisms used in cloning and LD50 data.
3. Experiment involving viral vectors potentially infect human cells and transgene likely toxic to human cells or growth.

สารชีวภาพ Risk group 3 / animal toxin group 2 จาก พรบ. เชื้อโรค

The following items are within the coverage of category 3

4. Experiment involving the transformation of animal embryos using viral components or genomes which may create or release a complete virion.
5. Experiment in which multidrug resistance genes that confer resistant to antibiotics currently used in human/animal/agriculture.
6. Transgenic plants that contain DNA from exotic infectious agents or contain genes coding for vertebrate toxins.
7. Those that can not verified risk category.

CATEGORY 4 VERY HIGH/ UNACCEPTABLE RISK HAZARD TO LABORATORY STAFF, COMMUNITY AND ENVIRONMENT AND/OR CONSIDERD IMMORAL IN PRINCIPLE

Do not allow

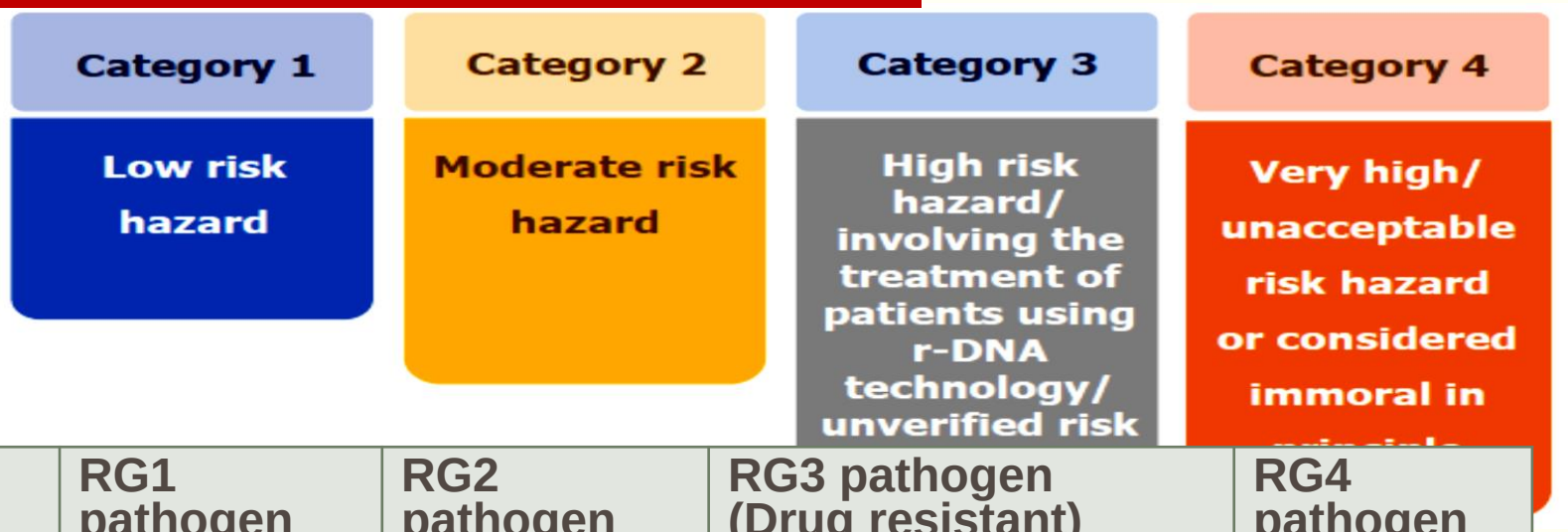
The following items are within the coverage of category 4

1. Those that using organisms classified in risk group 4 (see appendix B-IV - NIH guideline, 2016).
2. Experiment that lacking scientific certification and protective standards and/ or measure.
3. Experiment that aiming to produce infectious organisms and/ or toxins for warfare and genocide.

สารชีวภาพ Risk group 4/ animal toxin group 3 จาก พรบ. เชื้อโรค

Example only:

not definite depending on procedure



Status	RG1 pathogen	RG2 pathogen	RG3 pathogen (Drug resistant)	RG4 pathogen
Collect & kill directly on the spot	BSL1	BSL1	BSL1	BSL1 (Biosafety practice)
DNA or protein (non-toxin, harmless)	BSL1	BSL1	BSL1	BSL1
Collect living and extract DNA in Lab	BSL1	BSL1 plus BSC, autoclave	BSL1 plus BSC, autoclave, BSL2 practice or sometimes BSL2	Prohibited
Living	BSL1	BSL2	BSL3	Prohibited
Living > 10L/batch	BSL2	BSL3	BSL1	BSL1

สิ่งส่งตรวจ: คน สัตว์ สิ่งแวดล้อม

<u>สิ่งส่งตรวจ: คน สัตว์</u>

มีการ screen หาเชื้อโรค/ อาการโรคติดเชื้อ หรือไม่

- หาก <u>มี</u> จัดประเภทงานตามกลุ่มเสี่ยงของเชื้อโรคนั้นๆ
- หาก <u>ไม่มี</u> จัดเป็นงานประเภทที่ 2 เสมอ

<u>สิ่งส่งตรวจจาก สิ่งแวดล้อม</u> เช่น ดิน น้ำ ต้นไม้

มีการ screen หาเชื้อโรค หรือไม่

- หาก <u>มี</u> จัดประเภทงานตามกลุ่มเสี่ยงของเชื้อโรคนั้นๆ
- หาก <u>ไม่มี</u> มาจากแหล่งที่มีความเสี่ยงหรือไม่?
  - ดิน แหล่งใด ภาคกลาง ภาคอีสาน
  - น้ำดี น้ำเสียทั่วไป น้ำเสียโรงพยาบาล

BIOSAFETY LEVEL FOR MICROORGANISMS & MODERN BIOTECHNOLOGY

Level of Biosafety (BMBL guideline)

- Biosafety Levels 1-4 (BSL)

Microorganism/Modern Biotechnology or Genetic Engineering

- Increasing levels of employee and environmental protection
- Guidelines for working safely in research & medical laboratory facilities

Animal Biosafety Levels 1- 4 (ABSL or BSL-N)

Laboratory animal facilities

- Animal models that support research
- Guidelines for working safely in animal research facilities

Plant Biosafety Levels 1- 3 (BSL-P)

Plant laboratory, pilot house, experimental fields

Biosafety Guidelines

Risk Assessment

↓

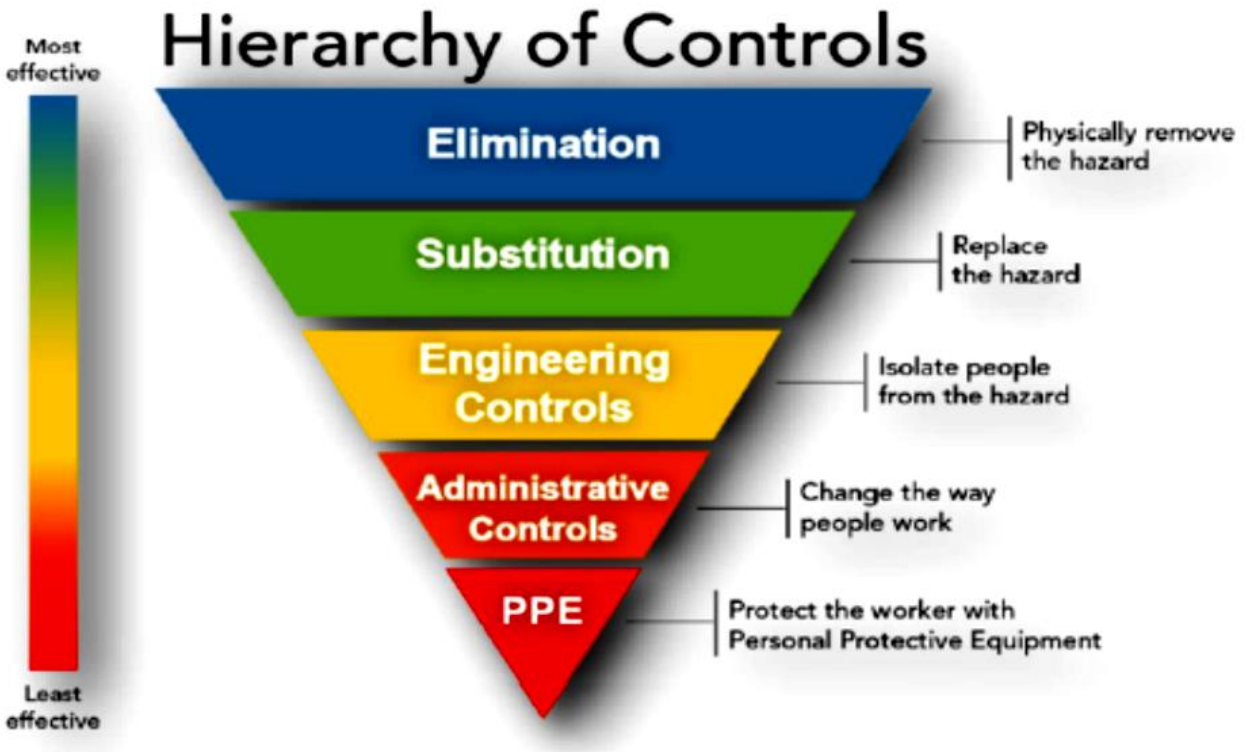
Classified risk
Category 1 - 4

↓

Containment

↓

Biosafety Level
1 - 4



Summary of Biosafety Concepts from the BMBL

Principles of Biosafety *(Based on risk assessment)*

1. Practice and Procedures
   - Standard Practices (Good microbiological practice)
   - Special Practices & Considerations

2. Safety Equipment (Primary barrier)

3. Facility Design and Construction (Secondary barrier)

4. Increasing levels of protection of items 1-4 (BSL1→BSL4)

Common components of Biosafety and Biosecurity

- Good laboratory practices
- Risk assessment
- Management oversight
- Personnel qualifications
- Control and accountability of organisms
  - Inventory management
- Access control
- Training
- Emergency Planning

  (some conflicts also exist)

APHL™ Analysis. Answers. Action.

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Practice and Procedures
1. Good microbiological practice

- **Most important concept / Strict adherence**
- Aware of potential hazard (risk assessment)
- Trained & proficient in techniques (risk minimization)
- Supervisors responsible for:
  - Appropriate Laboratory facilities
  - Personnel & Training
- Special practices & precautions
  - Occupational Health Programs

Standard Microbiological Practice

- Access to laboratory is controlled. Keep door closed while the laboratory is in session.
- Eating, drinking, smoking, using mobile phone, application of cosmetics, handling contact lenses and storing food is not permitted in lab areas.
- Do not playing around or using distracting electronic devices.
- Avoid loose fitting items of clothing, long hair must be restrained, do not wear dangling jewelry.
- Wearing appropriate personal protective equipment (PPE) and remove before leaving the laboratory.

Minimum – lab coat/gown, safety glasses, gloves

Standard Microbiological Practice

- Remove rings, watches or other sharp objects, including long nails before wearing gloves. Wash hands after all laboratory activities, after removing gloves, following contact with any hazardous agent, and before leaving the laboratory.
- Do not touch your face/hair or clean areas with gloves on.
- Mouth pipetting is prohibited.
- Minimizing the production of aerosols or working in a biological safety cabinet when aerosol production is unavoidable.
- Properly disposing of waste.
- Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant on a routine basis, and especially after spills, splashes, or other contamination.

Practice and Procedures

2. Special Practices & Considerations

- Know in advance what you are working with
- Risk group of pathogens and categories of modern Biotechnology activities

- Read available resources (Pathogen safety data sheet: PSDS)

  https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html

Pathogen safety data sheet: PSDS

SECTION I – INFECTIOUS AGENT
SECTION II – HAZARD IDENTIFICATION
PATHOGENICITY / TOXICITY
COMMUNICABILITY/ HOST RANGE/ INFECTIOUS DOSE/ INCUBATION PERIOD
SECTION III – DISSEMINATION
RESERVOIR/ ZOONOSIS / REVERSE ZOONOSIS / VECTORS
SECTION IV – STABILITY AND VIABILITY DRUG SUSCEPTIBILITY / DRUG RESISTANCE
SECTION IV – FIRST AID AND MEDICAL
SECTION VI – LABORATORY HAZARDS
SECTION VII – EXPOSURE CONTROLS AND PERSONAL PROTECTION
SECTION VIII – HANDLING AND STORAGE

What is the hazard in lab? → reduce exposure (action plan)

2. Safety equipment (Primary <containment> Barrier)

To minimize exposure of worker and Lab Environment to microbes (Prevent contact/ aerosols)

- **Personal Protective Equipment (PPE)**
  - ✓ Gloves, gowns, Respirator, Face shield, Booties
- **Engineering controls/ equipment**
  - ✓ Biological Safety Cabinets (BSC)
  - ✓ Covered or ventilated animal cage systems
  - ✓ Mechanical pipetting device
  - ✓ Safety centrifuge cups – Removable rotors
  - ✓ Autoclave

3. Facility Design and Construction
Secondary Barrier/ Engineering controls

Contributes to worker protection

Protects outside environment & Neighborhood

- **Building & Lab design,**
  - Separation of lab from public access
  - Restricted access zones
  - Autoclave facilities
  - Handwashing and eyewash facilities
  - Specialized ventilation systems (Directional airflow)
  - Cage wash facilities, etc.

4. Increasing levels of protection

Biosafety Levels (BSL) 1-4

- **Each level builds on previous levels**
- Increasing emphasis on safety procedures and practices
- Increasing need for training, preparation and competent supervision
- Increasing requirements for PPE and facility containment

Biosafety Level-1 (BSL-1 or ABSL-1 or BSL-P-1)

- **Group 1 agents (RG1) or Category 1 projects**

The organism(s) used in the experiment should be

- Non-infectious, especially to healthy adults, animal and plants.
- Present the least danger to the individual and the environment.

- Biosafety cabinet **not required** (unless creating aerosols)
- Require dedicated infectious waste bag and sharp bin

Practices

1. Standard Microbiological Practice and aseptic technique.
2. Work can be performed on an open lab bench or table.
3. Waste should be categorized and disposed properly.
4. Storage and working area of biohazard materials, genetically modified organisms and genetic materials must be labeled with the universal biohazard symbol and/or the word "biohazard".

Safety equipment

1. PPE that appropriate to the risk level such as laboratory coat, gloves and closed-toed shoes.

Facilities

1. There is no specific recommendation that the laboratory be isolated from other parts of the building. The lab should have doors to separate the working space with the rest of the facility.
2. Laboratories windows that open to the exterior should be fitted with screens.
3. Floors must be slip resistant. The laboratory should be designed so that it can be easily cleaned. Laboratory furniture must be capable of supporting anticipated loads and uses. Spaces between benches, cabinets, and equipment should be accessible for cleaning.
4. Bench tops must be impervious to water and resistant to heat, organic solvents, acids, alkalis, and other chemicals.
5. Laboratories must have a sink for hand washing
6. A sink and eyewash are readily available.

Biosafety Level-2 (BSL-2 or ABSL-2)

BSL2 practices applies to

- RG1 and RG2 pathogens
- Some activities of RG3* pathogens

Notification of the Ministry of Health
Entitle "the list of pathogens intended to be under the control under section 18"
B.E 2561 (2018)
http://blqs.dmsc.moph.go.th/assets/Bpat/PATratchakitcha182561.pdf

- Category 1 and Category 2 projects as well as specific aspects of Category 3.
- The organism(s) used in the experiment should present no greater than low to medium risk to healthy adults, animal and plants and low aerosol generation.

Biosafety Level-2 (BSL-2 or ABSL-2) practice/facility

- **BSL-1 facility <u>plus</u> the following**

- **Biosafety cabinet is required (proper installed)** or other approved containment devices (decontaminated daily)
- **Limited access** to lab when work in progress

✓Sink for hand washing (not operate by palm)

✓**Autoclave available in the same building**

✓**Biohazard Sign posted at entrance to lab**

✓**Label all equipment (incubators, freezers, etc.)**

Biohazard Sign at entrance of BSL-2 Lab.

- Separate from public area
- Lockable door.

- Name and telephone number of the responsible person(s)

http://blqs.dmsc.moph.go.th/page-view/176

- All wastes from laboratories and animal rooms are appropriately decontaminated before disposal.
  - Infectious material and waste transport by double package (Same building) or triple package (different building)

- Spills and accidents which result in overt exposures to organisms containing recombinant or synthetic nucleic acid molecules are immediately reported to responsible person. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.

Biosafety Level 2

- ✓**Documented training**
- ✓Baseline serology or pre-vaccination may be required

Biosafety Level 3 (BSL3)

BSL-3 practices applies to Category 3 projects, the organisms including

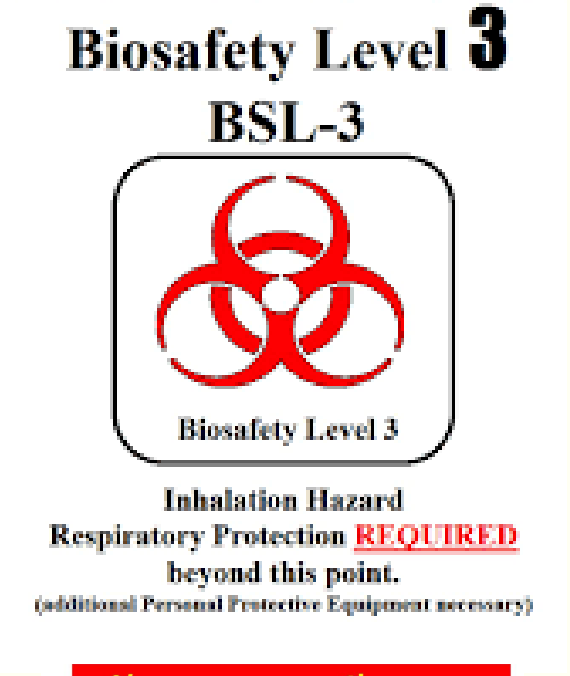
- Organisms that pose high risk to healthy adults, animal and plants.
- Airborne pathogens for virulent diseases

Group 3 (RG3) Agents

Primary hazards: needle sticks, ingestion, exposure to infectious aerosols

Biosafety Level 3 (BSL-3)
Working in High Containment

- Public access NOT permitted
- Biohazard Signs and labels posted
- Daily decontamination after spill and upon completion of experiment
- No sharps unless absolutely necessary
- **Autoclave install within laboratory**



EH&S Academy Brenda J. Wong, UCSD Biosafety Officer

Biosafety Level 3 (BSL-3)

Working in High Containment

- Primary barriers:
  - Similar to BSL-2 personal protective equipment ***plus***
  - Respiratory equipment if risk of infection through inhalation
- Wrap around disposable clothing is required. Specialized equipment may be required depending upon procedures
- **Bench top work not permitted**
- Documented training **and *personnel competency certification*** **(for BSL-3 procedures)**

UNC SCHOOL OF PUBLIC HEALTH
NORTH CAROLINA CENTER FOR PUBLIC HEALTH PREPAREDNESS

Biosafety Level 3 (BSL-3)

- Baseline serology
- Spills – report immediately and treat accordingly
- Vaccinations/post exposure protocols
- Biosafety Manual, Biosafety Officer

UNC SCHOOL OF PUBLIC HEALTH
NORTH CAROLINA CENTER FOR PUBLIC HEALTH PREPAREDNESS

Biosafety Level 4 (BSL-4)

- **Group 4 (RG4) Agents (Prohibited)**
- Primary hazards:
  - respiratory exposure to infectious aerosols
  - mucous membrane exposure to infectious droplets
  - accidental sticks with needles or other sharp objects contaminated with infectious material
- Personnel must receive ***specialized training*** in handling extremely dangerous infectious agents, containment equipment and functions
- Access to lab is restricted: immunocompromised persons are never allowed to enter the lab

Biosafety Level 4 (BSL-4)

- Standard practices include BSL-3 ***plus***:
  - strictly controlled access to the laboratory;
  - changing clothing before entering and exiting lab (showering upon exiting recommended);
  - decontaminating all material exiting facility

- Primary barriers:
  - Biosafety cabinets used at other biosafety levels
  - Full-body, air-supplied, positive pressure personnel suit

Protective suit with self-

ประกาศกระทรวงสาธารณสุข

เรื่อง การขนส่ง การส่งมอบ การทำลาย และการทำให้สิ้นสภาพเชื้อโรคและพิษจากสัตว์

พ.ศ. ๒๕๖๑

Notification of the Ministry of Health

Entitle "Transport and Dispose of Pathogen and Animal Toxin"

B.E 2561 (2018)

Standard of Transportation of Biomedical Materials by Commercial Air (National and International)

Amorn Upakaew
Biosafety Officer

USAMC-AFRIMS

OTHER CONSIDERATION

Group 1 (RG1) Low risk	**No restriction (appropriate/ safe)**
Group 2 (RG2) Moderate risk	- Triple packaging - Secondary receptacle is not restricted (omitted only for same building transport - Outer = endurance = total enclosed, cardboard, plastic or metal
Group 3 &4 (RG3) High risk	Triple packaging - Outer = UN recommendation Commercial
Animal Toxin (all groups)	Double package

Packaging Specification Marking

example: UN 4H"/Class 6.2/94 GB/2470

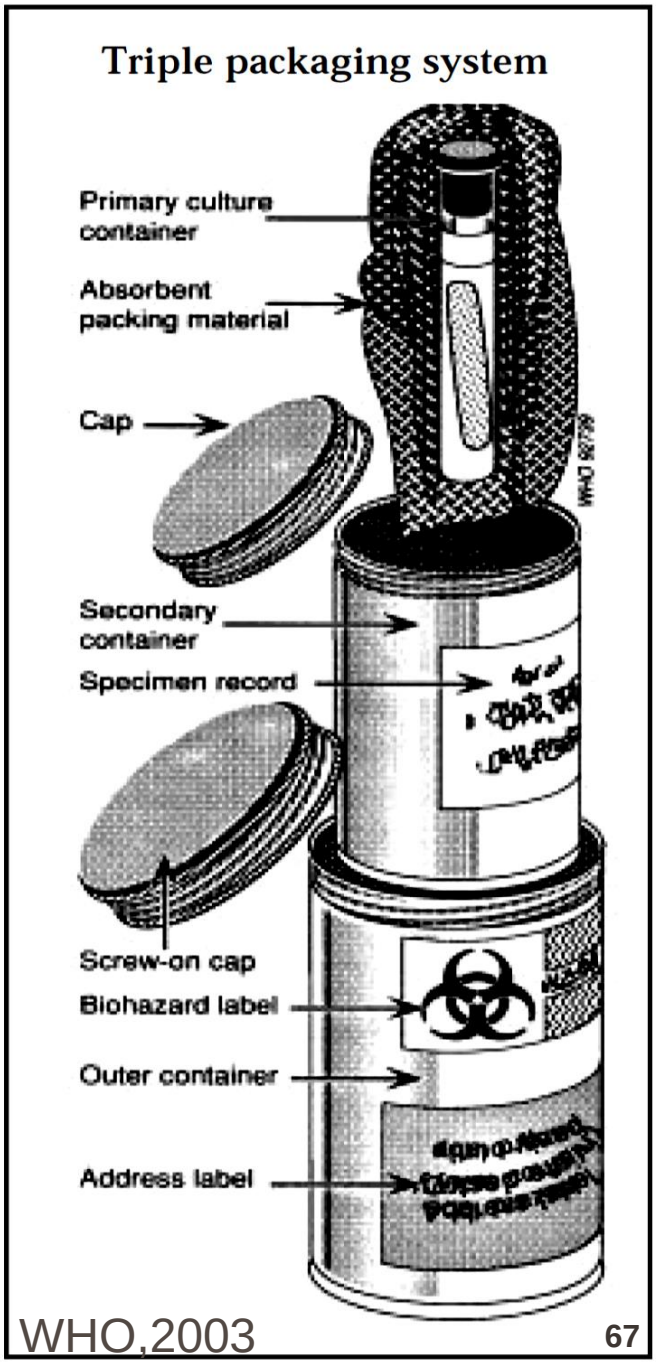
Transport within Thailand

Basic triple packaging system

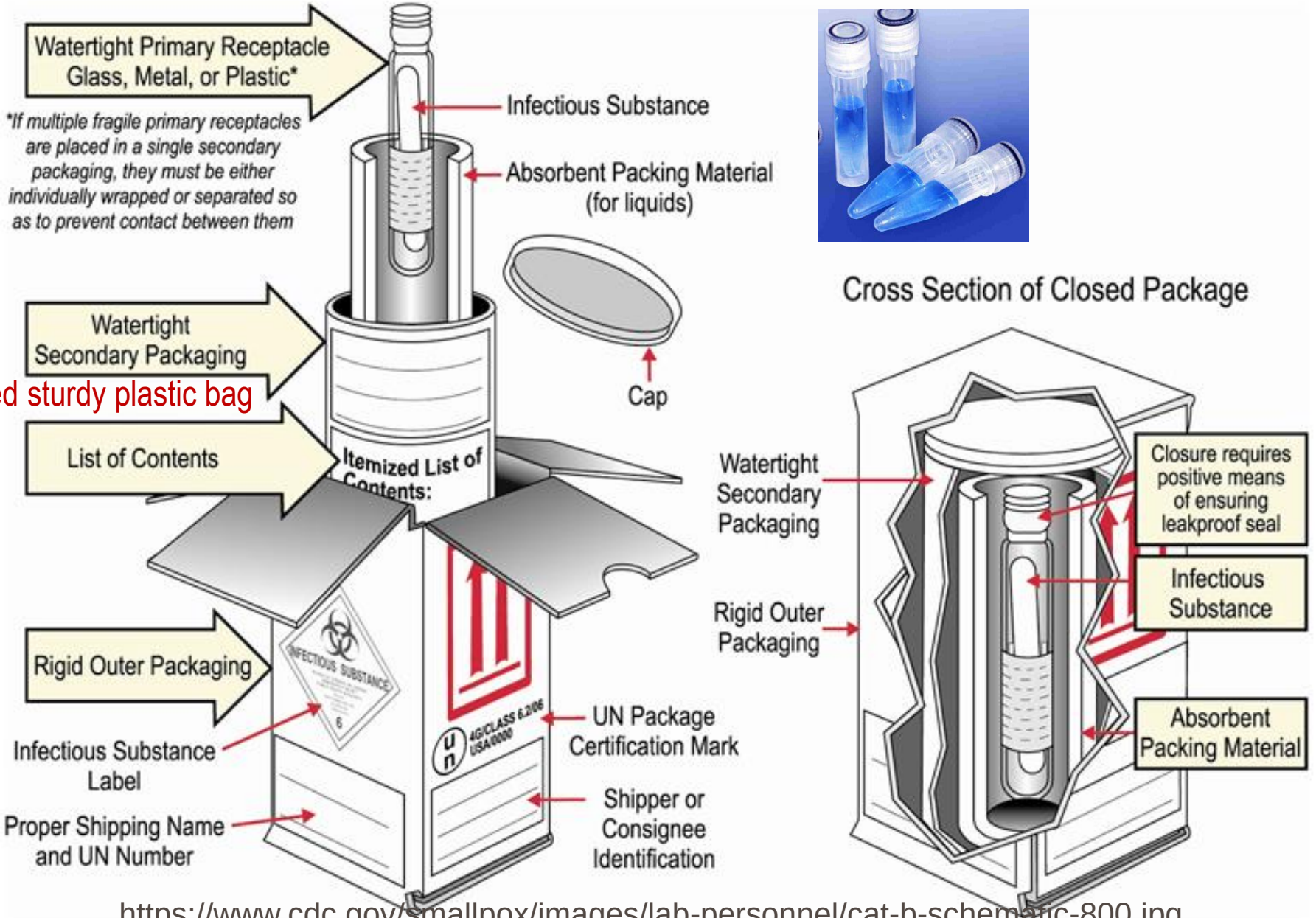
1. Primary receptacle. A labelled primary watertight, leak-proof receptacle containing the specimen. **The receptacle is wrapped in enough absorbent**

2. Secondary receptacle. A second durable, watertight, leak-proof receptacle to enclose and protect the 1° receptacle(s). **(having capacity to hold the total content of 1° receptacle)**

3. Outer shipping package. The secondary receptacle is placed in an outer shipping package which protects it and its contents from outside influences such as physical damage and water while in transit.



Commercial Triple packaging system



https://www.cdc.gov/smallpox/images/lab-personnel/cat-b-schematic-800.jpg

Transportation within Thailand (Different building)

- Triple packaging
- Caretaker with the knowledge on biosafety and emergency response
- Package is never leave alone
- Spill kit or disinfectant (suitable to the package volume)
- Vehicle
  - Institutional (personal vehicle is acceptable)
  - Non public transport
  - 4 wheels vehicle or bigger

https://asm.org/ASM/media/Policy-and-Advocacy/LRN/Sentinel%20Files/PackAndShip.pdf

WHO Module 3 Packaging of Infectious Substances . SHIPPERS' PROGRAMME 2015-2016

- Store used lab coat individually
- Store used lab coat in safe location (lab area)

- Clean lab coat can be folded and store outside lab area

BSL-2 lab coat

Front is not open, no side slit, no front pocket

BSL1 ?
Undergrad. Students?

Minimize Aerosols

- Don't
  - Use Bunsen burners
  - Drop liquids onto hard surfaces
  - Blow out last drop in pipette
  - Mix by suction + expulsion
  - Open centrifuge before it stops

Minimize Aerosols

- Do
  - Discharge liquid down side of container
  - Deliver as close as possible to contents
  - Use capped tubes when mixing or vortexing
  - Use care with needles (gauze pad with alcohol on septum of blood culture bottle)

Biosafety Cabinet

Emergency response: *Example only*

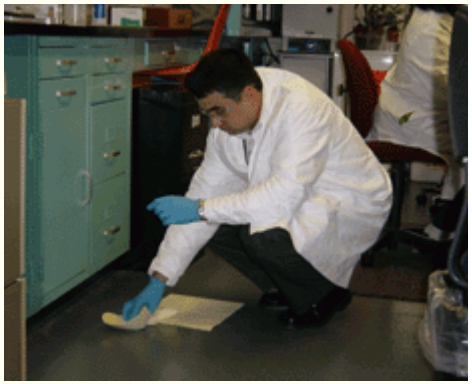
Biohazard Spill Kit

The biohazard spill kit should include, but are not limited to:

- A copy of the **Spill Cleanup Protocol**
- **PPE**: Disposable gloves, Lab coat(s), Safety goggles, N95 dust mask respirator(s), Disposable shoe covers (booties)
- **Absorbent material**, such as absorbent paper towels, granular absorbent material, etc.
- All-purpose **disinfectant**
- Autoclavable **bucket** for diluting disinfectant (this can be used to store the kit contents when not in use)
- **Tongs and/or forceps,** and/ or dustpan and hand broom or squeegee, etc. (for picking up broken glass or other contaminated sharps)
- **Sharps waste container**(s)
- Autoclavable **biohazard waste bags**
- Biohazardous spill **warning signs**

Emergency response: Example only

Accidental Spills



1. Evacuate area, alert personnel and let aerosols to settle
2. Don PPE; Cover with paper towels and apply bleach solution
3. Allow 15 – 20 min contact time
   - Wipe up working towards center
   - Use tongs if broken glass is involved
   - autoclave the waste

Emergency response: First Aid Measures

- Splash to Eye or Needle stick Injury
  - Rinse thoroughly for as much as possible (15 minutes!!!) at the eyewash or sink
  - **Call Occupational Medicine → Telephone number?? (signpost)**

Infectious waste management and decontamination

ถังขยะที่มีฝาปิด (ไม่ใช้มือเปิด)

Monday, April 22, 2019 Chonlaphat Sukasem, Ph. D.

เครื่องมือและอุปกรณ์ทำลายเชื้อโรค

Monday, April 22, 2019 Chonlaphat Sukasem, Ph. D.

การนึ่ง (autoclave)

121–132 ซ ความดัน 15 ปอนด์/นิ้ว^2
อย่างน้อย 15 นาที
ทำลายเชื้อจุลชีพทุกชนิด
รวมทั้ง bacterial spore
(ควรใช้เวลานึ่ง 1 ชม)

นิยมสำหรับขยะติดเชื้อทั่วไป

การอบร้อน (hot air oven)

160-170 ซ
เวลา 1-2 ชั่วโมง
ทำลายจุลชีพได้ทุกชนิด
รวมทั้งสปอร์แบคทีเรีย

Spore strips
Dry heat sterilization

เผาเป็นจุล (incineration)

800 -1000 ซ

ทำลายจุลชีพได้ทุกชนิด

นิยมสำหรับชิ้นส่วน อวัยวะ ซากสัตว์ทดลอง ขยะติดเชื้อที่ไม่นำกลับมาใช้

การต้ม (boiling)

100 ซ

ทำลาย bacteria, fungus และ virus ภายใน **10** นาที

ยกเว้น สปอร์ของแบคทีเรียทนได้นาน

Classification of chemical inactivate levels

High-level disinfectant:

ทำลายเชื้อจุลชีพทุกชนิด

ถ้าให้ระยะเวลาที่เหมาะ
อาจทำลายสปอร์แบคทีเรีย

Formaldehyde (gas)

Chlorine dioxide (gas)

Hydrogen peroxide (vapor)
Formaldehyge (gas)
Chlorine dioxide (gas)

อบห้อง

ตู้ชีวนิรภัย

Hydrogen peroxide
(vapor)

Intermediate-level disinfectant

- ทำลายเชื้อจุลชีพทั่วไป รวมทั้ง *M. tuberculosis*

Alcohol, Hypochlorite, iodine, idophore, phenolic compound

-ต้องคำนึงถึงระยะเวลาในการสัมผัสเชื้อ

-ระยะเวลาในการเตรียม

ใช้ในห้องปฏิบัติการทั่วไป

Low-level disinfectant

- ทำลายเชื้อก่อโรคทั่วไป ยกเว้น *M. tuberculosis*

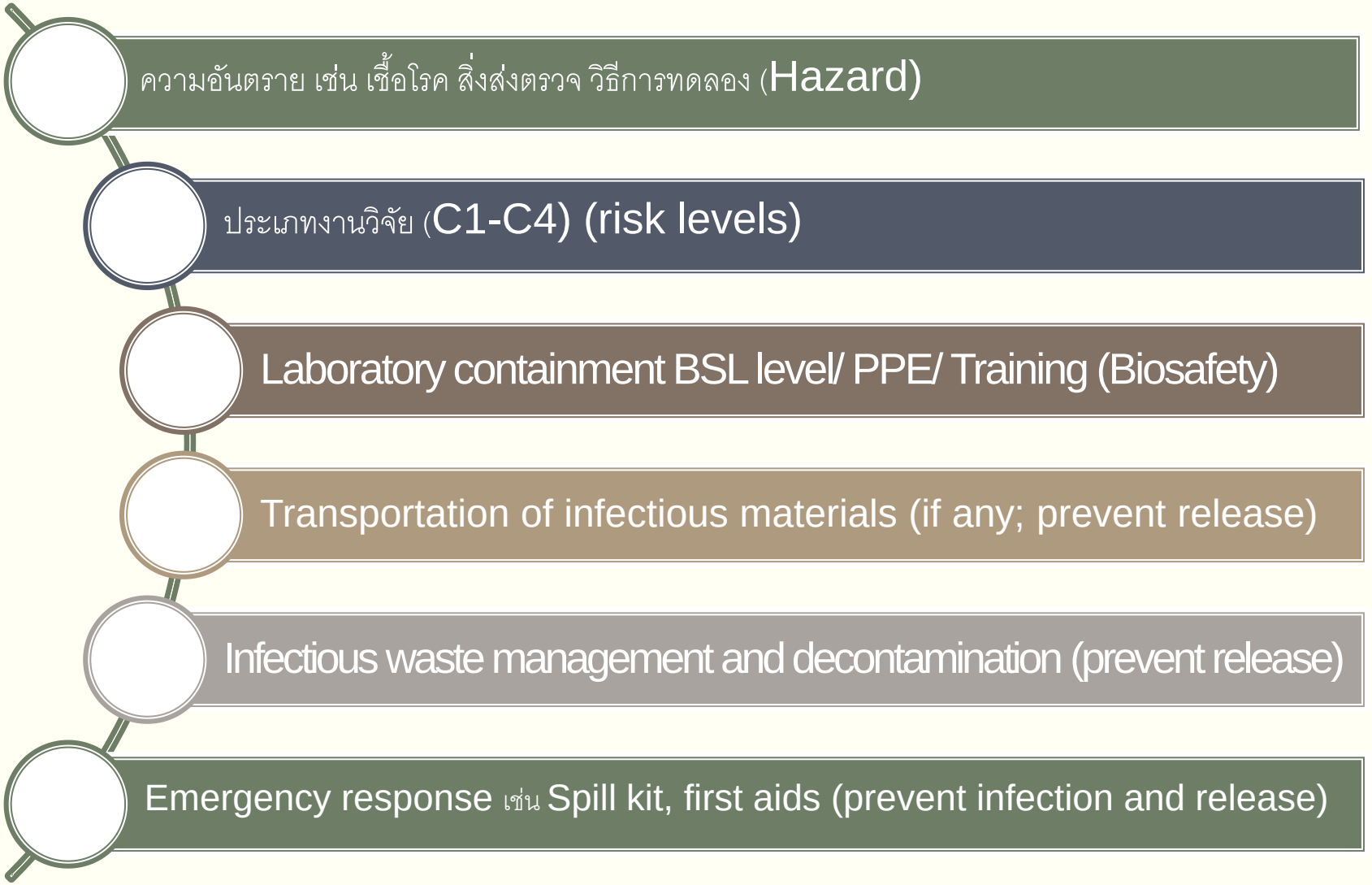
Quaternary ammonium compound

ใช้ในการเช็ดถูพื้นผิว

Summary

- Laboratorians have long recognized hazards of processing infectious agents
- Biosafety guidelines developed to protect workers in microbiological and medical labs through a combination of safeguards including engineering controls, management policies and work practices.
- Issue described differences between biosafety levels

ข้อมูลสำคัญที่ต้องชี้แจงให้ชัดเจนในการยื่นขอการรับรองให้ดำเนินการด้านความปลอดภัยทางชีวภาพ



Thank you for your attention.

Questions are welcome.