Comparative Study of Colorimetric DNA Hybridization Method and Conventional Cultural Method for the Detection of Salmonella in Frozen Chicken Meat and Frozen Seafood for Export

Aroon Bangtrakulnonth

Division of Clinical Pathology, Department of Medical Sciences

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Adherence Sites of Aeromonas Hydrophila in Human or Animal Intestines

Wantana Paveenkittiporn

Division of Clinical Pathology, Department of Medical Sciences

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By using formalin-fixed human or animal intestinal mucosa, we investigated the adherence sites of clinical isolated Aeromonas hydrophila. A. hydrophila strain produced cell hemagglutinins (HAs) which was detected by human erythrocytes, adhered well to the human ileal or colonic mucosa and displayed more strikingly adherence to the mucus coating the villus surface than to the epithelial cell durface. The adherence was D-manose and L-fucose sensitive, and was roughly correlated with the HA levels of the strain. Under the tested conditions, there were no significant differences between child and adult intestines, in term of adherence. Moreover, animal (porcine or rabbit) small intestines provided adherence sites that were comparable to (or even greater than) the human intestinal mucosa. Contrary to the above observations, a poor cell-associated HA producer displayed and extremely low levels of adherence to all the intestinal mucosa tested. The data suggested that the mucus coat covering human small intestine is the best adherence target for A. hydrophila and that cell-associated HAs may at least play a role in the adherence to human or animal intestines.

Ecology of Vector Mosquitoes in Relation to Epidemiology of Japanese Encephalitis in Thailand

Usawadee Thavara

Division of Medical Entomology, Department of Medical Sciences

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Monthly change on the JE-vector mosquito density and JE-virus infection rate in wild-caught mosquitoes were studied in 4 provinces with high and low incidence of Encephalitis during January to December 1991. The project was carried out once a month in 3 experimenal sites of each province. All-night light-trap collections were performed, trapped mosquitoes were separated by morphological charactors into vector species, sex and in the case of female, by blood feeding. Two hundred mosquitoes were pooled and kept in liquid nitrogen for JE-virus antigen detection by ELISA method. The results revealed that there were sharp increases of the vector mosquitoes and the infection of JE-virus in wide-caught mosquitoes before the occurrence of human cases in high incidence areas. The study suggested that monitoring of the vector density and the infection rate of JE-virus in wide-caught mosquitoes must be an important component of an early warning system in Thailand, and a standard simple method is required, however they are necessary to prove this hypothesis by longitudinal studies in all regions of Thailand.

④　タイ側提出の評価表

EVALUATION SHEET

DATE: 24 February 1992

This sheet should be submitted to the Evaluation Team by the Thai responsible persons of each Division of National Institute Health, Department of Medical Schiences as a basic document for a joint evaluation report that the Team would make with the Thai side.
Please check the following items and give us your comment on the National Institute of Health Project.

Your Division (VIRUS RESEARCH INSTITUTE)
Your profession ☑ Doctor (Professional division:) ☐ Nurse ☐ Paramedical ☐ Others
Achievement: 5-very good. 4-good. 3-fair. 2-not enough. 1-poor

	Achievement	COMMENT
1. Project management Japanese side Thai side	⑤ 4 3 2 1 ⑤ 4 3 2 1	-
2. Japanese experts (1) Long-term experts Contribution on the whole (2) Short-term experts Term Frequency Contribution on the whole	 5 ④ 3 2 1 5 ④ 3 2 1 5 ④ 3 2 1 5 ④ 3 2 1	-
3. Training in Japan Term Number of received persons Achievement	 5 ④ 3 2 1 5 ④ 3 2 1 5 ④ 3 2 1	-
4. Technology transfer (Write the items of technical transfer and tick the achievment of each item.) SEE IN "COMMENT"	 ⑤ 4 3 2 1 ⑤ 4 3 2 1 ⑤ 4 3 2 1 5 ④ 3 2 1 5 ④ 3 2 1 5 4 3 2 1	1. PCR for diagnosis dengue viruses. 2. PCR for serotyping of non-serotypible human rotavirus. 3. EIA for detection of Rabies NT. 4. Nucleotide sequencing technique for molecular epidemiology of influenza virus. 5. Preparation of dengue and Japanese encephalitis antigens.
5. Institute facilities Building	⑤ 4 3 2 1	-
6. Donated Materials Equipment	⑤ 4 3 2 1 5 ④ 3 2 1	-
7. Your Division as a whole	5 ④ 3 2 1	-
8. Do you have any problem in your Division ? If any. please describe in detail.		No.
9. Other comments if any		Long term training course in Japan, Thai participants which have family will successfully achieve training course if JICA supports the fund for their family visiting one time per course.

EVALUATION SHEET

DATE:

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Please check the following items and give us your comment on the National Institute of Health Project.

Your Division (Division of Clinical Pathology)
Your profession ☑ Doctor (Professional division: Director) ☐ Nurse ☐ Paramedical ☐ Others
Achievement: 5-very good, 4-good, 3-fair, 2-not enough, 1-poor

	Achievement	COMMENT
1. Project management Japanese side Thai side	5 4 ③ 2 1 5 4 ③ 2 1	Both sides should have frequently discussed on research projects under NIH programme.
2. Japanese experts (1) Long-term experts Contribution on the whole (2) Short-term experts Term Frequency Contribution on the whole	 5 ④ 3 2 1 5 4 ③ 2 1 5 ④ 3 2 1 5 ④ 3 2 1	 Expert was limited in number and field of studies required. Researchers (staffs) other than the counterpart have no opportunity to learn from the short-term experts
3. Training in Japan Term Number of received persons Achievement	 5 ④ 3 2 1 5 4 ③ 2 1 5 4 ③ 2 1	
4. Technology transfer (Write the items of technical transfer and tick the achievment of each item.)	5 ④ 3 2 1 5 ④ 3 2 1 ⑤ 4 3 2 1 ⑤ 4 3 2 1 5 4 3 2 1 5 4 3 2 1	- Biochemical and Biological Characterization of P. pseudomallei, C. difficile and Nocardia. - Immunofluorescent microscopy for the rapid diagnosis of melioidosis. - DNA-DNA hybridization for bacterial identification. - Technique for amplication of DNA by polymerase chain reaction.
5. Institute facilities Building	5 ④ 3 2 1	
6. Donated Materials Equipment	 5 ④ 3 2 1 5 4 ③ 2 1	 Provided computer (NEC) has a limited capacity and could not apply to the IBM system.
7. Your Division as a whole	5 ④ 3 2 1	
8. Do you have any problem in your Division ? If any, please describe in detail.		The researches on etiology and reference activity of infectious diseases have been going on well. But we still rely on using diagnostic reagents or test kits from developed countries.
9. Other comments if any		To be a self-reliance, reagents for using in laboratory diagnosis of national important diseases should be able to produce by NIH and distribute to other public health laboratories.

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DATE:

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Please check the following items and give us your comment on the National Institute of Health Project.

Your Division (Health Science Research Institute)
Your profession ☑ Doctor (Professional division:) ☐ Nurse ☐ Paramedical ☐ Others
Achievement: 5-very good. 4-good. 3-fair. 2-not enough. 1-poor

	Achievement	COMMENT
1. Project management Japanese side Thai side	5(4)3 2 1 5 4(3)2 1	
2. Japanese experts (1) Long-term experts Contribution on the whole (2) Short-term experts Term Frequency Contribution on the whole	5(4)3 2 1 5 4(3)2 1 5 4(3)2 1 5 4(3)2 1	
3. Training in Japan Term Number of received persons Achievement	5(4)3 2 1 5(4)3 2 1 5 4 3(2)1	Some participants did not work in the field that they were trained in Japan.
4. Technology transfer (Write the items of technical transfer and tick the achievment of each item.)	5(4)3 2 1 5(4)3 2 1 5(4)3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1	- Virus antivation of HIV - Immunofluorescence assay for HIV - PCR
5. Institute facilities Building	5(4)3 2 1	
6. Donated Materials Equipment	(5)4 3 2 1 (5)4 3 2 1	Materials and equipment donated for 3 laboratory and AIDS study have been very useful for its activities.
7. Your Division as a whole	5 4(3)2 1	
8. Do you have any problem in your Division ? If any, please describe in detail.		
9. Other comments if any		

EVALUATION SHEET

DATE:

This sheet should be submitted to the Evaluation Team by the Thai responsible persons of each Division of National Institute Health, Department of Medical Schiences as a basic document for a joint evaluation report that the Team would make with the Thai side.
Please check the following items and give us your comment on the National Institute of Health Project.

Your Division (Medical Entomology)
Your profession ☐ Doctor (Professional division:) ☐ Nurse ☑ Paramedical ☐ Others
Achievement: 5-very good, 4-good, 3-fair, 2-not enough, 1-poor

	Achievement	COMMENT
1. Project management Japanese side Thai side	 5 4 3 2 1 5 4 ③ 2 1	
2. Japanese experts (1) Long-term experts Contribution on the whole (2) Short-term experts Term Frequency Contribution on the whole	 5 4 3 2 1 ⑤ 4 3 2 1 5 4 ③ 2 1 ⑤ 4 3 2 1	
3. Training in Japan Term Number of received persons Achievement	 5 4 3 ② 1 5 4 3 ② 1 ⑤ 4 3 2 1	
4. Technology transfer (Write the items of technical transfer and tick the achievment of each item.)	⑤ 4 3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1	Entomology and epidimiology of JE.
5. Institute facilities Building	⑤ 4 3 2 1	
6. Donated Materials Equipment	 ⑤ 4 3 2 1 ⑤ 4 3 2 1	
7. Your Division as a whole	5 ④ 3 2 1	
8. Do you have any problem in your Division ? If any, please describe in detail.		We need more support in term of fellowships or experts.
9. Other comments if any		

EVALUATION SHEET

DATE:

This sheet should be submitted to the Evaluation Team by the Thai responsible persons of each Division of National Institute Health, Department of Medical Schiences as a basic document for a joint evaluation report that the Team would make with the Thai side.
Please check the following items and give us your comment on the National Institute of Health Project.

Your Division (Division of Biological Products)
Your profession ☐ Doctor (Professional division:) ☐ Nurse ☐ Paramedical ☑ Others
Achievement: 5-very good, 4-good, 3-fair, 2-not enough, 1-poor

	Achievement	C O M M E N T
1. Project management Japanese side Thai side	5 (4) 3 2 1 5 (4) 3 2 1	
2. Japanese experts (1) Long-term experts Contribution on the whole (2) Short-term experts Term Frequency Contribution on the whole	5 4 3 2 1 5 (4) 3 2 1 5 4 (3) 2 1 5 4 3 (2) 1	The experts we needed in some special fields were not available.
3. Training in Japan Term Number of received persons Achievement	5 (4) 3 2 1 5 (4) 3 2 1 5 (4) 3 2 1	
4. Technology transfer (Write the items of technical transfer and tick the achievment of each item.) Production of acellular pertussis vaccine. Production of PCEC rabies vaccine.	5 (4) 3 2 1 5 (4) 3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1 5 4 3 2 1	
5. Institute facilities Building	(5) 4 3 2 1	
6. Donated Materials Equipment	 (5) 4 3 2 1 (5) 4 3 2 1	
7. Your Division as a whole	5 (4) 3 2 1	
8. Do you have any problem in your Division ? If any, please describe in detail.		
9. Other comments if any		

⑤ ＮＩＨフォローアップ・プロポーザル案

Proposal for Follow-Up Period of Technical Co-operation for the Research Promotion Project in the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand

Background:

In April 1987, the National Institute of Health under Japanese Grant Aid Programme was officially opened. The Institute functions under the administration of the Department of Medical Sciences as the central organization for research activities to support the prevention, control and health promotion nationwide.

The 5-year Research Promotion Project, under the Japanese technical co-operation through JICA, has been implemented from August 1985 through July 1990. Most objectives of the Project were successfully achieved except some activities of which the scope of work has not been completely carried out yet, and thus the technical cooperation for the Research Promotion Project has been extended for 2 years, i.e. from August 1, 1990, through July 31, 1992.

Objectives of the First Five-year Project:

1. To promote research necessary to control infectious diseases by introduction of new technology.

2. To cooperate in the research of developing biological products necessary for the control of the infectious diseases prevailing in Thailand.

3. To established the infrastructure of such facilities commonly for various relevant activities, as an animal experiment, a scientific instrument center, an RI laboratory and a biohazard laboratory.

4. Other activities mutually agreed upon as necessary.

Achievement of NIH Activities:

From August 1, 1985, through July 31, 1990, with the cooperation of the Japanese Government and the endeavour of the respective personnel of both Thai staff and Japanese experts, many activities have been satisfactorily achieved and progressed. In brief it could be summarized as follows:-

1. Vaccine Development. Four vaccines were developed. JE vaccine was 95% achieved, it has passed through all steps of production and the field trial shows high potency and safety. Rubella and rabies vaccines were 50% achieved, while acellular pertussis vaccine was 40% achieved.

2. Reference Activities. The reference activities in NIH were promoted in the areas of bacterial and viral identification, typing, contribution to epidemiology and insect reference museum.

3. Study in Virology. Many aspects which were studied, such as immunity in AIDS, Herpes and HFRS; epidemiology of Rota, Influenza and JE; and diagnosis of HIV infections.

4. Development of Diagnostic Kits. Some diagnostic kits were developed, such as RPHA kit for HBsAg, Dengue test kit and Rubella test kit, RIA kit for prevention and control of iodine deficiency disorder.

5. Establishment of Common Laboratories. Animal experimental center, Scientific equipmnet center, Radioisotope laboratory and P3 laboratory. These laboratories serve both activities in NIH and in other organizations.

6. Establishment of new laboratories . Besides, immunology laboratory, mycoplasma and rickettsiology laboratories have been established.

7. Study in Medical Entomology. The JE vector surveillance was developed by detection of mosquito density and JE virus antigen in wild caught mosquitoes.

NIH has presently been recognized by many internal and external organizations as a training center. It hosted international training courses, and provided individual and group training to overseas participants.

During the extension period, many activities have been rapidly progressed , which can be summarized as follows:-

1. Vaccine Development. JE vaccine was completely achieved, and the Government Pharmaceutical Organization is now producing the vaccine in large scale to support the country need. Rabies vaccine as well as acellular pertussis vaccine were 75% achieved, both have passed through all production steps but still need further improvement. The field trials are to be performed.

2. Reference Activities. The reference activities in NIH were promoted in the area of bacterial and viral identification, insect reference museum, and biological products control and laboratory service for poliomyelitis to support the national and international polio eradication programme. The Virus Research Institute will be recognized as the WHO Regional Reference Center.

3. Study in Virology. Many aspects were studied, such as epidemiology of HIV, Rota, Influenza, Dengue and JE (especially the HIV study, NIH had cooperated with Japan and CDC of the United State), immunity in AIDS, JE and Poliomyelitis.

4. Study in Bacteriology and Mycology. The diagnostic methods and diagnostic tools of some infectious diseases have been studied. Several laboratories have been established, and various tests for infectious diseases have been launched, i.e. mycoplasma laboratory, the laboratory for study the pattern of Phage type of Staphylococcus aureus, and I.D. test for systemic candiasis with reagents.

5. Development of Diagnostic Kits. Some diagnostic kits are being under developed, such as ELISA kit for rapid diagnosis of Melioidosis, ELISA kit for detection of IgM/IgG antibodies of dengue infection and Japanese Encephalitis. Some Diagnostic kits are going to be developed, such as ELISA and RIA kits for Hepatitis B and respiratory syncytial virus infections.

6. Strengthening of immunology, biotechnology, radioisotope laboratories, scientific equipment and animal experiment centers to serve the activities in NIH as well as in other organizations.

7. Study in Medical Entomology. The density and seasonal change of JE vector mosquitoes in different regions, insecticide resistance of mosquito vector, as well as asthma causing agent are being studied.

Justification for the Follow-Up Period of Technical Cooperation for the Research Promotion Project:

As stated above, the present project has gained much success in each individual subject of research activities. But, it should be emphasized that the most important contribution was the development of human resources at NIH in answering to the Objective of the Project "the promotion of research capabilities". Many Thai counterparts were stimulated and encouraged in their science activities. Many of them proceeded to higher trainings such as master course in the universities and some others are now under Ph.D. course in Japan.

However, the project still has some items of cooperative research which are promising, but still need further support for their completion. Besides, with the rapid socio-economical development of Thailand, the Thai people are now confronted with more health problems than before including AIDS and other infectious diseases, and the responsibility of NIH is increasingly greater.

In the Steering Committee Meeting held on July 17, 1991, therefore, a proposal was made that a follow-up period of Technical Co-operation for the Research Promotion Project be necesssary for another 2 years as a real impact to the health status of Thai people.

Objectives for the two-year Follow-Up Period:

1. To bring the promising but still incomplete subjects of research activity to a successful conclusion by cooperation with Japanese experts, especially in the field of the control measures for infectious diseases (biological products, diagnostic microbiology and immunology, epidemiology).

2. To implement the wider use of diagnostic kits which have been developed in the past years of the project.

3. To strengthen the role of NIH as national and international reference centers for infectious diseases including AIDS so that "the third country training programme" will be expected to start in future with the function of a South-East Asia Reference Regional Center.

Plan of Operations:

1. The Follow-Up Period for Technical Co-operation will be for two years, starting from August 1, 1992, through July 31, 1994.

2. One of the Deputy Director-Generals of DMS will serve as the Director of NIH.

3. The Co-ordinating and Steering Committees will be organized for the effective operations of the Project.

4. Thai and Japanese scientists will jointly conduct research work in selected subjects for the control of infectious diseases as shown on the attachment.

Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
I. Dengue Hemorrhagic Fever	- Serodiagnosis (HI, ELISA) - Virus isolation (T.C., IF) - DNA Diagnosis (PCR)	- Study the possibility of PCR as a tool for early diagnosis	Number: 1 Duration: 1 month		Number: 1 Duration: 1 year Subject: Molecular biology of Flavivirus	
II. Acquired Immune Deficiency Syndrome	- IFA test kit was produced and served for confirmation test - Virus isolation - PCR	- Study biological and immunological properties of HIV strains isolated in Thailand	Number: 1 Duration: 3 months			
III. Melioidosis	- ELISA test for Melioidosis - Partial purification of exotoxin	- Production of monoclonal Ab - Gene cloning	Number: 1 Duration: 1-3 months Field: Production of monoclonal Ab	Number: 1 Duration: 1-3 months Field: Genecloning		

Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
IV. Viral Hepatitis B	- Epidemiology of Hepatitis B - Development of RPHA reagent - HBs Ag was produced from recombinant vaccinia virus and used in ELISA for anti HBs detection - DNA probe was produced from recombinant plasmid and used in setting up dot blot hybridization for hepatitis DNA detection	- Development of Hepatitis B ELISA Kit - To increase the sensitivity of hepatitis DNA detection, PCR Technique - HBV genone replication in order to synthesize specific primers, gene sequence analysis which correlates to our probe should be investigated	Number: 1 Duration: 1-3 months Field: Development ELISA Kit of Hepatitis B Virus	Number: 1 Duration: 3 months Field: Set up PCR technique including sequencing, analysis of probe and primer synthesis	Number: 1 Duration : 1 year year : 1993 Field: Recombinant DNA technology	

Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
V. Diarrhea						
- Viral diarrhea	- Isolation of rotavirus in cell culture - Serotype of rotavirus by ELISA serotyping and PCR method - Study on molecular property of isolated rotavirus by ds RNA electropherotyping, RNA-RNA hybridization	- Molecular sequence of unusual strains and cultivate non serotypable of rotavirus - Study on other causative agents of viral diarrhea such as enteric adenovirus type 40,41 and other small round virus		Number: 1 Duration: 1-3 months		
- Bacterial diarrhea	- Detection of Salmonella by commercial kit	- Production of diagnostic kit (DNA hybridization)		Number: 1 Duration: 1-3 months		

Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
VI. Acute Respiratory tract infection	- Virus isolation (Egg, T.C.) - Identification - ARI surveillance	- Development of diagnotic kits by using monoclonal antibody against RSV - Genomic characterization of the virus isolated from ARI children	Number: 1 Duration: 1-3 months			
VII. Rabies	- Development of PCEC rabies vaccines	- Field trial of PCEC rabies vaccines - Production of rabies vaccine in SPF quail cell culture by rolling method	Number: 1 Duration: 3 months Field: Rabies vaccine production by rolling method		Number: 1 Duration: 1 year Subject: Preparation of antigens, antisera and testing for extraneous viruses	

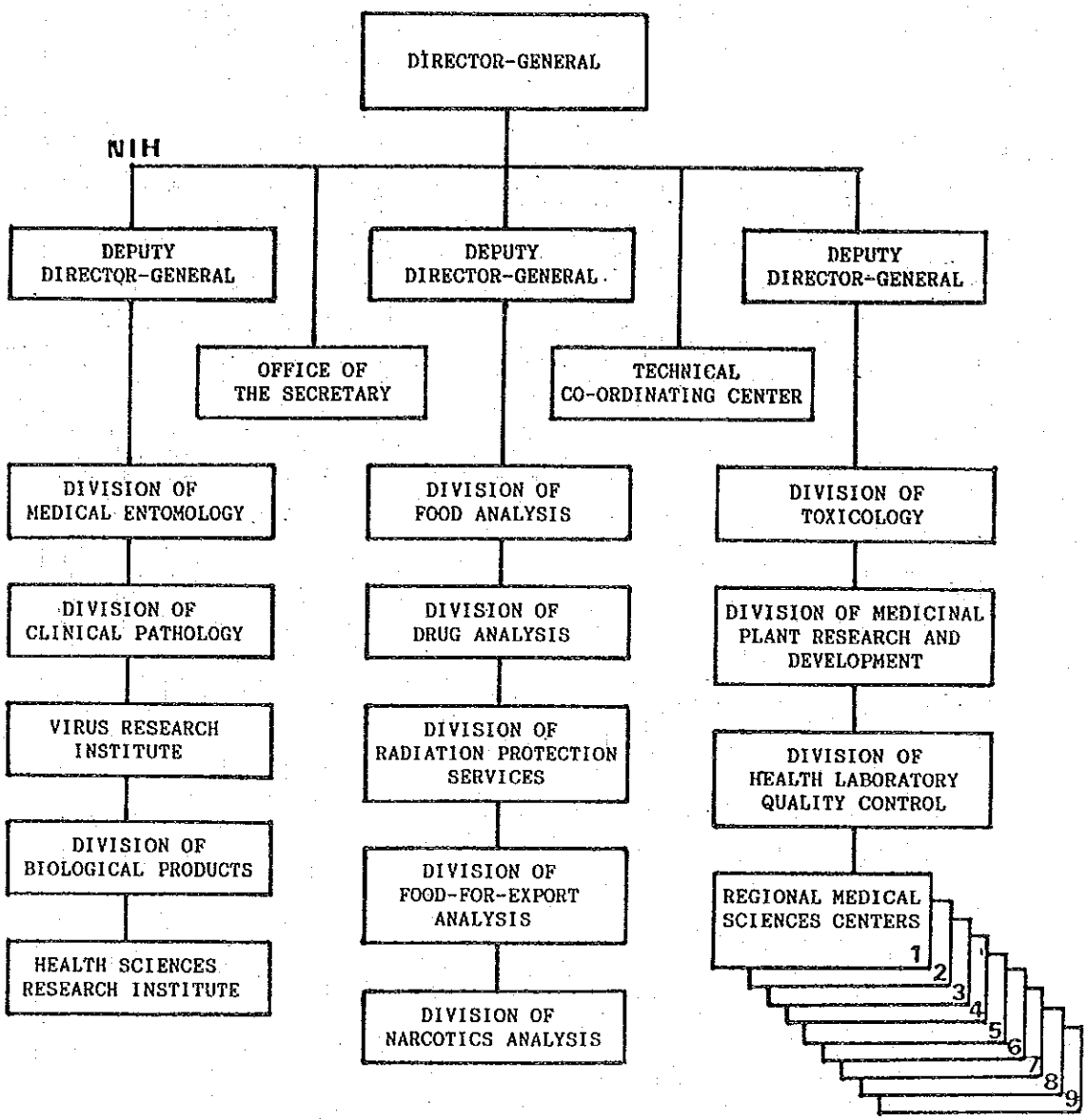
Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
VIII. Diptheria		Improvement of diptheria toxoid production in fermentor	Number:1 Duration: 3 months Field: Diptheria toxoid production in fermentor			
IX. Herpes genitalis Herpes zoster	The extract from medicinal plants (Clinacanthus nutous) have been determined antiviral activities against HSV2 and VZV by plaque reduction assay and dot blot hybridization. The extract with high activity has been	It is necessary to study the mechanism of this extract, how it inhibits the viral growth or interact with each virus. The mechanism will be studied by comparing protein patterns and structure of viruses between	Number:1 Duration: 3 months			

Diseases (Public Health Importance)	Achievement of Activities	Future plan	Expert needed		Fellowship needed	Equipment
			1993	1994		
	produced into cream base and has been used in treatment with herpes genitalis and herpes zoster	viral growth in treated and non treated cells with the extract.				
X. Monitor mouse colony						
- Sendai viral pneumonia	Detected by HI, CF and ELISA using commercial Ag and Ab.	To produce Ag and Ab	Number:1 Duration: 2-3 months		Number : 1	
- Mouse hepatitis	Detected by CF and ELISA using commercial Ag and Ab	To produce Ag and Ab				

⑥　ＤＭＳ及びＮＩＨ組織図

ORGANIZATION CHART

DEPARTMENT OF MEDICAL SCIENCES



ORGANIZATION CHART
NATIONAL INSTITUTE OF HEALTH