タイ国国立衛生研究所プロジェクト
評価専門家チーム調査報告書

平成４年３月

国際協力事業団
医療協力部

タイ国国立衛生研究所プロジェクト
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序　文

本プロジェクトは、タイ国内に流行する各種の感染症、寄生虫症等の対策を講じるために、これら分野の研究能力の向上及び人材の養成を目的として、昭和60年８月１日から５年間にわたり開始されたものである。

プロジェクトは概ね順調に進捗、当初協力期間中に基礎的な部分の技術移転についてはほぼ当初の目標を達成したと評価されたが、研究促進のためには、なお日本の協力の継続が必要との結論に基づき、さらに２年間にわたり協力期間が延長され、現在に至っている。

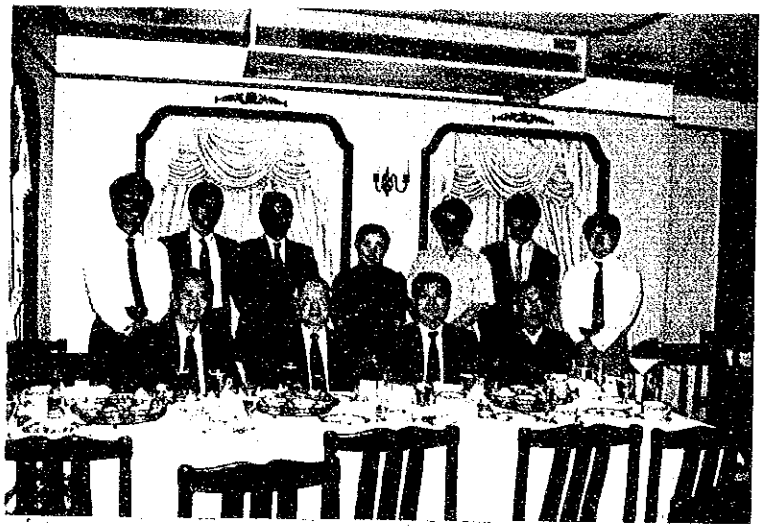
今般、上記延長期間が平成４年７月末に終了するのを前に、その間のプロジェクトの実績、活動状況をタイ側とともに評価し、あわせてタイ国立衛生研究所・National Institute of Health（ＮＩＨ）の今後の方向について協議することを目的として、徳永徹国立予防衛生研究所所長以下の評価専門家チームを派遣した。

本報告書は、上記チームが行った調査、タイ側との協議等の内容及び結果をとりまとめたものである。

ここに、本件調査実施に当たりご協力いただいた関係各位に対し、深甚なる謝意を表するとともに、今後とも本件技術協力の成功のために一層のご支援とご協力をお願いする次第である。

平成４年３月

国際協力事業団
医療協力部長
曽　我　紘　一



日本側関係者

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団長主催のレセプション

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1. 評価専門家チーム派遣

1.1 派遣の経緯と目的

タイ国国立衛生研究所プロジェクト（以下プロジェクトと略す）は、タイ国政府が自国内に流行する各種感染症、寄生虫症等に対処するための衛生研究活動を行う研究機関の設立について、我が国に無償資金協力及び技術協力を要請してきたことを受けて開始されたもので、1984、1985両年度にわたり無償資金協力により建物建設及び機材供与（1984年度24.5億円、1985年度14.6億円）を実施した。これに時期を合わせる形で、1984年7月プロジェクト方式技術協力のための事前調査団を派遣、更に1985年4月実施協議調査団を派遣し、同年8月から5年間にわたる協力を開始した。

プロジェクトの目的は、①タイ国内に流行する感染症に係る研究能力の向上、②これら感染症の抑圧に必要な生物製剤の研究開発、③各部門間の共同利用施設の利用体制の強化、等である。

当初協力期間終了に先立ち、1989年12月プロジェクトの実績評価のための調査団を派遣、プロジェクトの活動状況、当初目標の達成状況等についてタイ側との間で協議・検討した結果、以下のような結論に至った。

当初のマスタープランに沿って達成度を評価すると、第一は感染症部門、医昆虫部門の基礎的技術移転は終了し、技術を応用・展開する点が残されている。次に、生物製剤部門では、日本脳炎ワクチンで実験室レベルの協力をほぼ終了しており、タイ側が実際にワクチンを生産する段階である。第三に、狂犬病ワクチンでも、当初プロジェクト協力期間終了時までに野外実験に移り、80%程度まで終了させることができる。風疹ワクチン、百日咳ワクチンでは、実験室レベルの協力がかなり進んでいるが、これらワクチンの製造段階が残されている。これらの評価を総合すると、各分野とも基礎レベル技術移転はほぼ終了段階を迎えているが、その応用・展開としての研究は開始されたばかりであり、特に上記のうち、ワクチンの試験製造に関しては風疹ワクチン、百日咳ワクチンの技術移転が協力期間内では達成でき得ない状況である。（以上、平成2年2月タイ国立衛生研究所プロジェクト評価調査団報告書より引用）

この評価結果を踏まえて、研究技術の応用、自主的な研究テーマの選定、タイ側スタッフ相互間の研究交流促進等について、さらに2年間わたり協力期間を延長することで双方の間で合意し、1990年8月1日から2年間わたり協力を延長することとなった。

延長期間に入って以後も本プロジェクトの協力は順調に推移、タイ側におけるプロジェクトに対する評価も極めて高いものがある。

本件評価専門家チームは、以上のような背景のもとに、現行協力期間の終了を約半年後に控えた1992年2月末、延長期間内におけるプロジェクトの実施状況及び実績の評価を目的として派遣された。

派遣に先立ち、プロジェクトのタイ側カウンターパートに対し質問票を送付、あらかじめ回答を準備しておくよう要請した。また特に本プロジェクトについては、タイ側からすでに2年間のフォ

ローアップ実施要請が保健省から首相府技術経済協力局（ＤＴＥＣ）に提出されていることもあり、日本側におけるプロジェクトの今後の方向性検討の材料とするため、タイ側の考えるＮＩＨの将来計画について、詳細を聴取することとした。

1.2　専門家チーム構成

担　当	氏　名	所　属
団長（細　菌　学）	徳永　　徹	国立予防衛生研究所長
		ＮＩＨ国内委員
団員（ウイルス学）	高橋　理明	大阪大学微生物病研究所名誉教授
		ＮＩＨ国内委員
団員（ウイルス学）	山西　弘一	大阪大学微生物病研究所教授
団員（計画評価）	青木　利道	国際協力事業団医療協力部医療協力課課長代理
団員（協力計画）	奥田　久勝	国際協力事業団医療協力部医療協力課職員

1.3　調査日程

日順	月日	曜日	移　動　及　び　業　務
第１日	2.26	水	・移動（成田 ―――→ バンコク／ＴＧ６４１） （徳永・奥田） ・移動（大阪 ―――→ バンコク／ＴＧ６２１） （高橋・山西）
２日	2.27	木	・ＪＩＣＡ、日本国大使館表敬・打ち合わせ ・ＮＩＨ表敬・打ち合わせ カウンターパートからの聞き取り ・移動（成田 ―――→ バンコク／ＴＧ６４１） （青木）
３日	2.28	金	・ＤＭＳ局長表敬 ・ステアリング・コミティー開催 ・カウンターパートによる研究成果発表
４日	2.29	土	・合同評価レポート案検討等、チーム内打ち合わせ
５日	3. 1	日	・資料整理
６日	3. 2	月	・保健次官表敬（於：保健省） ・ＮＩＨにて、カウンターパート研究成果発表 ・合同評価レポート案につきタイ側と協議
７日	3. 3	火	・ＤＴＥＣ表敬、調査結果等につき協議 ・合同評価レポート署名 ・ＪＩＣＡ事務所・日本国大使館報告
８日	3. 4	水	・移動（バンコク ―――→ 成田／ＴＧ７６０） （徳永） ・移動（バンコク ―――→ 成田／ＴＧ６４０） （青木・奥田） ・移動（バンコク ―――→ 大阪／ＴＧ６２１） （高橋・山西）

1.4　主な面会者

(1)　タイ側

①　Ministry of Health

Dr. Uthai Sudsukh	Permanent Secretary

②　Dept. of Medical Sciences（DMS）

Dr. Preeya Kashemsant	Director General
Dr. Chongdee Wongpinairat	Director, Technical Coordinating Center

③　NIH

Dr. Nadhirat Sangkawibha	Honorable Consultant
Dr. Sompop Ahandrik	Director (Deputy Director General, DMS)
Dr. Chuinrudee Jayavasu	Principal Medical Officer
Dr. Mayura Kusum	Director, Div. of Clinical Pathology
Dr. Paijit Warachit	Director, Virus Research Institute
Mr. Prakong Phan-urai	Director, Div. of Medical Entomology
Ms. Kanchana Leelasiri	Director, Div. of Biological Products
Dr. Jakkriss Bhumixawasdi	Director, Health Sciences Research Institute

その他スタッフ

④　DTEC

Ms. Thipsuda	Chief, Japanese Sub-division

その他スタッフ

(2)　日本側

金井　興美	NIHプロジェクト・チームリーダー
中島　衡平	NIHプロジェクト調整員
稲垣　富一	DTEC派遣JICA専門家
長門　利明	在タイ日本国大使館一等書記官
阿部　信司	JICAタイ事務所所長
森　　千也	JICAタイ事務所所員

2. プロジェクトの概要

専門家チームは、２月27日ＮＩＨを訪問、徳永、高橋、山西各メンバーがそれぞれの担当専門分野の実験室を訪れ、タイ側カウンターパートの活動状況を調査するとともに、彼らとの間で種々の協議を行った。

翌28日午前、ＮＩＨにおいてプリーヤーＤＭＳ局長を表敬するとともに、同席したソムポップＮＩＨ所長、ナディラＮＩＨ名誉顧問ほかのＮＩＨ首脳を交えて討議を行った。その後引き続き、評価専門家チーム全員、金井リーダー以下ＮＩＨ派遣専門家チーム、森ＪＩＣＡタイ事務所員の出席のもとにステアリング・コミティーを開催、席上プロジェクト協力期間延長後現在までの活動状況について検討を重ね、プロジェクトの実績についての評価を行った。またあわせてＮＩＨの今後の方向についてタイ側の考えが明らかにされ、これについても意見が交換された。同コミティーの議事次第は附属資料の通りである。

28日午後及び３月２日は、以下のような内容についてカウンターパートからスライド、ＯＨＰを使用した研究報告がなされ、専門家チームとの間で質疑応答が行われた。

（研究報告テーマ及び発表者名）

- Serotyping of Nonserotypable Human Rotavirus by PCR Typing
  : Yaowapa Pongsuwanna
- The Complete Nucleotide Sequence of a Variant of Coxsackievirus A24
  : Kasama Supanaranond
- Molecular Epidemiology of Influenza Viruses
  : S. Pattamadilok
- Molecular Diagnosis of Dengue Viruses Using RT-PCR Technique
  : S. Rojanasuphot and S. Vongcheree
- Preparation of Dengue and JE Antigens for Antibody Capture ELISA
  : S. Rojanasuphot and S. Pothipunya
- Enzyme Immuno Assay for the Detection of Rabies Neutralizing Antibody
  : P. Samuthananon
- An I&M ELISA for the Serodiagnosis of Poliovirus Infection
  : Kasama Supanaranand and Osamu Nishio
- Biological and Immunological Study of HIV Isolated in Thailand
  : Wattana Auwanit
- Microbiological Monitoring of Laboratory Animals
  : raywadee Butraporn
- Development of Pertussis Component Vaccine
  : Teeranart jivapaisarnpong, Prayute Buddhirakkul
- Production of Chick Embryo Cell Culture Rabies Vaccine
  : Prakorb Ruenrairatanaroj
- Application of immunofluorescent microscopy to rapid diagnosis of melioidosis
  : Pimjai Naigowit
- Immunofluorescent microscopy for the detection of IgM and IgG antibodies to Pseudomonas pseudomallei
  : Pimjai Naigowit

- Extraction of bacterial lipopolysaccharides from P.psedomallei for immunodiagnosis of melioidosis by ELISA : Pimjai Naigowit
- Purified toxins from Clostridium difficile and related organisms : Siripan Wongwanich
- 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
- Adherence sites of Aeromonas hydrophila in human or animal intestines : Wattana Paveenkitiporn
- Ecology of vector mosquitoes of Japanese encephalitis in thailand : Usawadee Thavara

これらの結果に基づいて、合同評価報告書原案を作成し、双方で議論を交わし、意見を調整しつつ内容修正を行い、最終的な合意に達した後、3月3日評価専門家チームの徳永団長とプリーヤーDMS局長との間で上記報告書の署名・交換を行った。（附属資料①）

双方合意した結論の要点は以下の通りである。

(1) 全般的に見て、2年間の技術協力の延長に際して取り決められたR／Dの条項に従い、NIHにおける研究推進のプロジェクトは順調に進行しつつあり、大部分の目標は期間内に確実に達成できる見通しが得られた。

(2) しかしながら、一部の課題については必ずしも延長期間内に達成しうる見通しが得られず、さらに継続支援が必要と認められる。

(3) NIHは、エイズ及びエイズ関連疾患を含む感染症に対して、国のレファレンス・センターとしての役割を持っているが、この機能についてはかなりの前進が見られるものの、いまだ不十分であり、さらに強化する必要が指摘される。

(4) 「第三国研修」に関しては、東南アジアにおけるレファレンス地域センターの機能を持つ研修機関として、近い将来にそのプログラムを具体化し、発足させることが望ましい。

(5) このプロジェクトで得られたモダン・テクノロジーを、タイにおける他の医科学領域へさらに拡大応用するよう努力すべきである。

3. タイ側との協議内容

3.1 ステアリング・コミティーにおける討議内容

ステアリング・コミティーは、2月28日(金) 午前9時45分から昼過ぎまでＮＩＨ第Ａ－203会議室において、ソムポップ所長を議長として開催された。

(1) 会議は前述の議事に沿って進められ、まず双方の出席者が紹介された後、金井リーダーが附属資料②、(1)に基づいてＮＩＨプロジェクトのこれまでの活動状況について発表、さらに中島調整員が、2年間のプロジェクト延長期間における日本側の投入実績について附属資料②、(2)に基づいて発表した。

金井リーダーの発表に関する質疑応答の中で Dr.Nadhiratは、ＮＩＨのリサーチ活動についてコメントし、活動の向上はまだ不十分であると指摘、「スタッフの50％はコピーの段階」であり独自のリサーチ活動ができるようにする必要があると強調した。

(2) 続いてＮＩＨ側が、附属資料②、(3)に基づいてプロジェクト延長以後現在までのＮＩＨの業績について説明した。この説明に関する質疑応答の中で、我が方から日本脳炎ワクチンの製造状況について質したところ、ＧＰＯ(Government Pharmaceutical Organization)での同ワクチンの製造は成功しており、現在生産量は20万ドースに達し、順調に進んでいるとのことである。また狂犬病ワクチンのフィールド・トライアル実施計画については、マヒドン大学がＷＨＯの予算を得て行う予定であり、実施時期は未定であるとの回答があった。

なお Dr.Nadhiratから、上記資料②、(3) 26頁の 8.1.3 Characterization of HIVisolated in Thailand については、現在のところタイでは実施しておらず米ＣＤＣで行っているので、この部分を削除したいとの発言があった。

(3) ＮＩＨ側からさらに、92／93年におけるＮＩＨの活動計画について説明があった。上記資料②、(3)の中に示された内容は、当該年度におけるＮＩＨのすべての活動について述べたものであり、フォローアップ要請の内容とは直接関連するものではないとのことである。

タイ側の説明によれば、活動計画の中で最重点事項はエイズを含めた感染症のリサーチである。特にエイズ患者の日和見感染のサーベイについてタイ側の関心が強く、タイと日本の2か国のみではなく、他の機関等を含めた合同によるエイズ・ワクチン及び医薬品のリサーチを行いたい意向である。

また、これまで再三にわたり日本側から提案していながらタイ側が関心を示さないため全く動きのなかった第三国研修の実施について、積極的に実施したい意向が表明された。タイ側は、第三国研修の実施によりタイがラオス、カンボディアのような国を助けることができると述べ、すでにヴィエトナムを含めたアジア地域内の各国から研修のための研究者を受け入れている実績もあることから、ＮＩＨを利用した第三国研修の実施にむけて準備を進めたいとの意向を示した。

我が方からは、時期的にはこれからでは92年度中おける研修の実施は困難であると思われるが、いずれにしても手続きの詳細についてＪＩＣＡタイ事務所に照会するよう伝えた。

(4) 議事の最後として、チームが用意したジョイント・エバリュエーション・レポート案の検討を行った。

チーム側からレポート案の内容について説明、その後質疑応答に入った。実績等については特に先方からコメントはなかった。タイ側は、フォローアップの実施の可能性について我が方からの具体的な回答を求めてきたが、我が方は今回のチームが実施について正式にコミットする権限を与えられていないため、明確な回答は控えたいこと、したがってレポートの結論部分の記載もフォローアップの実施を明確にはしないことを伝えた。

我が方はしかしながら、日本側の総意として何らかの形でＮＩＨに対する協力は継続することを考えていることを明らかにし、チーム帰国後、今回の調査の結果を踏まえて日本側関係者の間ではどのような形での協力が望ましいかを検討すると述べた。

レポート案のうち、Accomplishment及びConclusionについては、さらに双方で協議することとした。

3.2 ＤＴＥＣとの協議内容

3月3日（火）午前、ＤＴＥＣ日本課長の Ms.Thipsuda及び同課スタッフとの協議が、ＤＴＥＣ内会議室において行われた。この協議には、ＤＴＥＣに派遣中の稲垣専門家も同席した。要旨は以下のとおり。

(1) ＤＴＥＣ側より、保健省からはすでに13プロジェクトの申請が提出されており、そのうち今回のＡＩＤに関する要請は1月のＪＩＣＡミッションの来訪を受けて、また食品衛生についても日本側のサジェストを受けて提出されたものであるとの認識が示された。

(2) 今回の評価に関連して以下の質疑応答が行われた。

1) ＮＩＨのAdministrationについて特に問題となる点はあるか。（ＤＴＥＣ）

－特に問題はないと思う。日本側の認識として、ＮＩＨは優良なプロジェクトであると評価できる。

2) ＡＩＤＳとの関連から見た場合、ＮＩＨの機材、人材及びAdministrationについて問題はあるか。（ＤＴＥＣ）

－ウィスル部門の人材を増やす必要があると思われる。

－人員増については、タイ政府の方針により困難であり、個人の能力を高めることが求められよう。（ＤＴＥＣ）

3) ＮＩＨがＷＨＯ等のサポートのもとに第三国研修を実施しているが、この点についてどのように考えるか。（ＤＴＥＣ）

－第三国研修は効果的と思われるので、日本側としてもサポートしたい。

－現在ＪＩＣＡベースの第三国研修は５コース行われており、他にもプロポーザルが多く、ＮＩＨの第三国研修がいつ実施できるか、見込みをつけることは困難である。（稲垣）

(3) ＤＴＥＣ側から、ＡＩＤＳ要請に関しては、３月までにＤＴＥＣから日本側に出したいとの意向が表明された。

(4) 食品衛生プロジェクトに関して、ＤＴＥＣ側から、かつて同種のプロジェクトをオランダに要請したことがあるが、実現しなかった経緯があることが明らかにされた。

(5) ＤＴＥＣ側から、ＮＩＨに供与されている高額機材について、保守等のサステナビリティーをどのように考えるかとの質問があり、我が方から、他のプロジェクトでも同様の問題があり、ＪＩＣＡとしても問題の重要性は認識していること、また現在の技術協力の枠の中の対応としては、プロジェクト終了から２～３年後に実施するアフターケアの制度があることを伝えた。

(6) 日本のフェローシップに２種類あるが（ＪＩＣＡベースと学術振興会ベース）、ＤＴＥＣとしてはチャンネルを１つにまとめて欲しいとの希望が表明された。

4. 各分野の評価

プロジェクト協力期間中における主要な業績は、下記の表のようにまとめられる。

本評価専門家チームは、ステアリング・コミティーにおける総括報告、２度にわたるカウンターパート研究業績発表、研究現場視察時における聞き取り調査等の結果をもとに、分野別評価を行った。

各専門家の報告分担は次のとおりである。

細菌学関連（徳永）、ウイルス学関連（高橋）、医昆虫学（徳永）、生物製剤の開発・改良（高橋）、遺伝子工学（山西）、発癌物質等（山西）、食品汚染（徳永）、実験動物（高橋）、中央機器、ＲＩ及びＰ３ラボラトリー（山西）

4.1 細菌学関連

(1) 嫌気性菌

下痢原性嫌気性菌として知られる Clostridium difficileの毒素に関する研究が中心で、本期間中もっとも進展を見た研究の一つとして考えられる。カウンターパートは、Mrs. Siripan Wongwanichで、本年度日本研修の予定であるが、既にオーストラリアに３年留学の経歴を持ち、本プロジェクトの事務局を担当している。下痢便中の本菌の検出率は細菌学的手法によっては4.8％であるが、毒素検出法によって52.5％と高く、さらに交叉反応性を除くため、近藤瑩子専門家と共同で、毒素の精製を進めており、硫安沈殿とＤＥＡＥ－セファロースカラムクロマトグラフィーを用いて４つの主要なピークを得て、各々に対する抗体作製も検討されている。

(2) メリオイドーシス

Pseudomonas pseudomalleiにより惹起されるメリオイドーシスはタイの全地域で見られる感染性風土病の一つであるが、敗血症の83.7％が死亡する重篤疾患である。その迅速診断法の確立を目指し、倉田　毅専門家の指導により、Mrs. Pimjai Naigowitが現在近藤瑩子専門家と共同で免疫蛍光抗体法による診断法を開発中である。18例のメリオイドーシスのサンプル中15例が陽性であり、他方非メリオイドーシスの44サンプル中６例が陽性ないし疑陽性であった。一方間接蛍光抗体法により、多数のメリオイドーシス患者を含むヒト血清を調べた結果、ＩｇＭ及びＩｇＧ抗体の測定が鑑別診断及び病期の診断に有用である可能性が示された。またP. pseudomalleiから抽出したＬＰＳによる免疫診断法の開発が、近藤専門家の指導で進行中である。ＬＰＳは同菌外膜主構成成分であり、精製ＬＰＳによるＥＬＩＳＡ法は迅速診断法として有用性が高いと考えられる。この分野からは、日・タイ共同で多数の原著論文が出されている。

(3) 腸管感染性細菌

カウンターパートの１人Mrs. Surang Dejsirilertは、既に1986年1年間の日本技術研修を終

了しており、現在岐阜医大江崎博士の指導を受け、細菌の同定、特にグラム陰性非発酵性菌を中心にＰＣＲ法の応用を担当している。チフス菌、大腸菌等種々の病原腸内細菌やPseudomonas pseudomalleiのＰＣＲ診断も試みている。 Miss. Orn-anong Ratchtrach enchaiは、1992年度日本研修予定者の１人であるが、３年前北里研　壇原博士及び順天堂大　山本博士よりＰＣＲの指導を受けたことがあり、大腸菌のキットの作成などの研究に意欲的である。

Mrs. Wantana Paveenkittinpornは、順天堂大　山本博士のもとに、昨年１年４ヶ月間研修留学した。現在フォルマリンで固定したヒト及び動物の腸管粘膜を用い、臨床分離されたAeromonas hydrophilaの結合部位の研究を行っている。種々の知見が得られているが、日本で研修した Pathogenicityの研究と、病院やフィールドで得られる菌株の同定との間のギャップは、カウンターパートに共通した問題意識の一つらしい。ＷＨＯのサルモネラ・シゲラセンターの担当者Mrs.Aroon Bangrakulnonthは、現在も北里研　壇原博士から指導を受けており、ＤＮＡハイブリダイゼーション法を用い、輸出用の凍結鶏肉や凍結魚肉からのサルモネラ検出などの研究を行っているが、調査団訪問中不在であり、詳細の情報は得られなかった。

(4) ファージタイピング

五箇所の病院において種々の病巣から分離された 463株のStaphylo coccus aureusについて、23種のファージを用いたタイピングを行った。カウンターパートはMiss Ren Sunthadvanichが中心であり、予研中村明子博士の指導を受けている。ファージタイプは３Ａ／３Ｃ／55／71（グループⅡ）が Naharaj Nakhon Ratchaburi病院株の25.6％を占め、他の４病院では、85型、53／77型および29／53／77／84型がそれぞれ33.5％、39％および15％であった。

チフス菌、パラチフスＡ菌のファージタイプもタイ国全土から集められる多数のサルモネラ株について行われており、結果は各地方へ還元され、レファレンスセンターとしての機能を果していると評価される。

(5) 真菌

医学博士 Vinita Brirajが真菌部門を担当しており、Candida albicans症、Penicillosis、Nocardiosis、Histoplasmosisなどについて幅広く同定法などの検討を行っている。とくにＡＩＤＳの蔓延に伴い cryptoco ccosis、aspergillosis、candidiosis等の症例が増加し、この部門の重要性が増加しつつある。またアフラトキシンなど、食品関係の真菌が注目されつつある。カウンターパートの１人Mrs. Natteewan Poonwanは、日本研修を終了し、病理学的面で予研倉田専門家の指導を受ける一方、現在近藤専門家と共にnocardiaの化学的分類法の研究を行っている。

4.2 ウイルス学関連

(1) インフルエンザウイルス

1990、1991年と根路銘専門家の努力によりインフルエンザウイルスの分子疫学の研究が行わ

れてきた。カウンターパートはMs. Sirima PattamadilokでＲＮＡ抽出後逆転写酵素を用い、ｃＤＮＡを作製し、遺伝子配列を決定する手法が出来るようになった。今後のインフルエンザウイルスの進化等の分子疫学の発展が期待される。

(2) エイズ（ＨＩＶ）

タイ国に於けるエイズキャリアーの数は年毎増加の一途をたどり1992年には40,000にもなるといわれている。1986年以来日本側の専門家と Mr. Wattanaを中心としたタイ側の努力でＭＴ－４細胞に感染させた抗原を用いての蛍光抗体法がＮＩＨのみならず、各分所でも用いられ重宝されてきた。1991年度には診断のための講習会が行われ約 200人もの人が参加し、地方への技術移転が行われた。

更に1990年度には日本のＡＩＤＳ財団の援助もあり、武部専門家にＰＣＲ法の手法を教わり米国ＣＤＣ、武部氏と共にタイ国のウイルス株の特徴を分子生物学的手法で解析し、タイには２種類（米国型とアフリカ型）が混在することを見いだした。1991年には生田専門家の指導でウイルス分離の技術の移転が行われ、数種のウイルス株分離が成功し、将来の更なる解析が期待される。

(3) ロタウイルス

谷口専門家により導入されたロタウイルスの分子生物学的分類法を用いて Ms. Yaopa Pongsuewannaを中心としたグループが研究を行っていて、バンコック及びソンクラで採取されたウイルスを分類している。単クローン抗体を用いてのサブグループへの分類のほかに、型特異的プライマーを用いてのＰＣＲ法によりアガロースゲル中の移動度の差により容易に分類が可能になった。その結果採取された便中のウイルスの中に新たなグループのロタウイルスが見いだされた可能性があり、今後の研究成果が期待される。

(4) 肝炎ウイルス

ＨＢウイルス、ＨＣウイルスによる肝炎は重要な問題ではあるが延長期間中は特に技術の交流は無かった。

(5) フラビウイルス

五十嵐、森田両専門家の努力でデングウイルスの分子生物学的手法によりデングウイルスの型別が可能になった。方法はＲＴ－ＰＣＲ法で少ない時間で判定が可能となり、今後の臨床材料への応用が期待される。更にデングウイルス、及び日本脳炎ウイルスの抗体捕捉ＥＬＩＳＡ法の開発のためのウイルス抗原作製も進みつつある。

(6) ＡＨＣウイルス

Ms. Kasama Supanaranond が予研での研修中に予研のグループと行った研究を帰国後もその研究を発展させている。研究の内容は急性出血性血膜炎の原因ウイルスの一つであるコクサッキーＡ24の変異株（ＥＨ24／70株）の全遺伝子配列を決定して、他のウイルスの塩基配列を比較するとポリオウイルスやコクサッキーＡ21と類似していることが判明した。

(7) ポリオウイルス

ポリオウイルス診断を行うための手段としてＩｇＭＥＬＩＳＡ法の開発を行い、ポリオウイルス感染を疑われる臨床材料の検索を行うと微小中和法より感度が高いことが判明し早期の診断に役立つ可能性がある。

4.3 医昆虫学

標本室の拡充・整備は完成し、媒介動物（蚊・ハエ・ダニ・ネズミ）の生態研究も進捗している。また媒介動物の制御に関しても、化学的制御法のほか、生物学的制御法の開発も試みられており、この部門は全体にまとまりも良く活発である。とくにタイにおける日本脳炎の疫学と関連してのベクター蚊の生態に関しては、1991年の１月から12月にかけて、日本脳炎発生率の高い地区と低い地区、計４地区について、それぞれ３箇所の蚊捕集実験区を設け、ＥＬＩＳＡ法などにより詳細調査の結果、ヒトの日本脳炎発生に先だって媒介蚊の急激な増加とウイルス感染が認められることが明かとなり、ベクター密度とベクターへのウイルス感染状況の調査により、流行可能なことが示唆された。さらに広範な調査と迅速簡便法の開発が必要であろう。

4.4 生物製剤の開発・改良

(1) 組織培養狂犬病ワクチンは順調に原液が生産され、また供与機材による設備の充実と技術の指導により濃縮の操作と精製の技術も定着するに至っている。力価は日本製のものとかわらない高品位であり、他のすべての基準も満たしている。カウンターパートは Prakorb Ruenrairatanarojが中心であり、化血研　坂本専門家の指導を受けた。

(2) 風疹ワクチンは研究室段階ではウイルスの増殖、力価測定は可能となっている。

(3) 百日咳無細胞ワクチンについてはＬＰＦ－ＨＡタイターの向上がまだ不十分である。カウンターパートは Teeranart JirapaisarnpongとPnayute Buddhirakkulであり、予研岩佐専門家の指導を受けた。

4.5 遺伝子工学

新たにＮＩＨにHealth Sciences Research Instituteが創られそのうち後記のサービス部門の他はImmunogy及び Biotechnologyの部屋が活動をしている。ImmunologyでＡＩＤＳの研究が行われていてウイルス分離、血清診断、遺伝子の解析が行われようとしている。一方 Biotechnologyの部屋ではMs. Kruavon Balachndraを主任に抗ウイルス剤としての医薬用植物の検索が行われ、抗ヘルペス効果のある物質を検索している。その他遺伝子工学的手法を用いて診断材料用のＨＢｓ抗原の作製法が始められようとしている。この部門はスタッフも若く今後の研究成果が期待される。

4.6　発癌物質等

延長の期間中には特に技術の交流はなかった。

4.7　食品汚染

食品汚染は、主として細菌学的側面から遂行されており、化学薬品による汚染は本プロジェクトに含まれない。したがって4.1(1)(3)(4)(5)を参照。

4.8　実験動物

実験動物室は日本側専門家の適切な指導と、タイ側カウンターパートの努力により非常にうまく維持運営されており、他の部門へのサービスも良好である。

4.9　中央機器、ＲＩ及びＰ３ラボラトリー

中央機器センターではコンピュターをはじめ各種の機器の維持及び修理が順調に行われている。ＲＩ実験室では各研究グループがＲＩを用いて研究の場にしている以外にバセドー病をはじめＲＩを用いての診断材の開発を行っている。Ｐ３実験室は主としてＨＩＶの分離及び診断材の準備に用いられ有効に利用されている。

5. 専門家チームの提言

タイ国は、東南アジア地域において我が国にとり最も重要な国の１つであり、またＮＩＨは、日本がこれまで７年間にわたりハード及びソフトの両面にわたり全面的な支援を行ってきた研究機関である。

ＮＩＨの今後の活動については、これまでの我が国の協力を基にした応用研究のさらなる活発化を期待するところであるが、環境問題の深刻化に代表されるタイ国内の経済活動の活発化に伴う諸問題に対処するために、ＮＩＨの役割がさらに重要視される状況になりつつあることを考えると、将来にわたり我が国の支援を継続し、かつ強化することを基本方策とすべきであろう。

本プロジェクトに関しては、タイ側から２年間のフォローアップ要請が提出されており、チームとしては我が国が基本的にこれを受け入れることを望むものである。

さらに、我がチームのバンコク滞在中に、対エイズ戦略に関する要望が各方面からあったが、日本政府として早急にこれに関する対策を立て、平成４年度中に協力を開始するとともに、可能な限りその協力の推進拠点をＮＩＨに置き、将来的にはエイズ対策のためのプロジェクトの中に現行のＮＩＨプロジェクトを吸収発展させ、一貫したＮＩＨ強化策として長期的に発展させることが、現実に日タイ双方にとり有益であると考える。

なお、今回現地においては、食品の品質管理に関する要望や議論はほとんど行われなかったが、この面でのＮＩＨの役割は大きなものがあり、将来はこの面からのＮＩＨ支援も考慮すべきものと考えられた。

附 属 資 料

①　合同評価レポート

JOINT EVALUATION REPORT

ON

JAPANESE TECHNICAL COOPERATION

FOR

THE RESEARCH PROMOTION PROJECT IN THE NATIONAL INSTITUTE OF HEALTH

IN

THE KINGDOM OF THAILAND

March 3, 1992
Bangkok
The Kingdom of Thailand

Mutually attested and submitted

to all concerned

Bangkok
The Kingdom of Thailand
March 3, 1992

Dr. Tohru Tokunaga
Leader,
Japanese Evaluation Team,
Japan International Cooperation Agency,
Japan

Dr. Khunying Preeya Kashemsant
Na Ayuthaya
Director General,
Department of Medical Sciences
Ministry of Public Health
The Kingdom of Thailand

Discussion meeting between the Evaluation Team of the Japan International Cooperation Agency (JICA) and the National Institute of Health, Department of Medical Sciences, for evaluation of Japanese Technical Cooperation for the Research Promotion Project in the National Institute of Health

Date : February 27 - March 3, 1992

Place : National Institute of Health, Department of Medical Sciences, Nonthaburi, The Kingdom of Thailand

Attendants : JAPANESE PANEL

JAPANESE EVALUATION TEAM

Dr. Tohru Tokunaga	Leader
Dr. Michiaki Takahashi	Member
Dr. Koichi Yamanishi	Member
Mr. Toshimichi Aoki	Member
Mr. Hisakatsu Okuda	Member

JICA Thailand Office

Mr. Senya Mori

Japanese Expert Team

Dr. Komi Kanai

Dr. Eiko Kondo

Mr. Kohei Nakajima

THAI PANEL

Dr. Nadhirat Sangkhawibha

Dr. Sompop Ahandrik

Dr. Chuinrudee Jayawasu

Dr. ML. Ratanasuda Phan-urai

Dr. Jakkriss Bhumixawasdi

Dr. Paijit Warachit

Mr. Prakong Phan-urai

Mr. Kamol Sawadimongkol

Mrs. Kanchana Leelasiri

Dr. Mayura Kusum

Mrs. Siripan Wongwanich

Dr. Chongdee Wongpinairat

I. INTRODUCTION

The Japanese Evaluation Team (hereinafter referred to as "the Team") organized by the Japan International Cooperation Agency (hereinafter referred to as "JICA") and headed by Dr. Tohru Tokunaga, visited the Kingdom of Thailand from February 27 to March 3, 1992 in order to jointly evaluate with the Thai authorities concerned the past achievements and future prospects of Japanese Technical Cooperation for the Research Promotion Project in the National Institute of Health (hereinafter referred to as "the Project") on the basis of the Record of Discussions concerning Extension of the Period of Japanese Technical Cooperation signed on July 31, 1990.

During its stay in the Kingdom of Thailand, the Team discussed and studied together with the Thai counterpart personnel concerned a number of aspects regarding the progress and achievements of the Project, as well as fulfillment of commitments.

Through careful studies and discussions, both sides summarized their findings and observations as described in the following chapters.

II. METHOD OF EVALUATION

1. Materials used as reference

In order to evaluate the past performance and achievements both quantitatively and qualitatively, the following materials were used as references:

(1) The Record of Discussions concerning Extension of the Period of Japanese Technical Cooperation
(2) The Tentative Schedule of Implementation
(3) The official requests made by the Government of the Kingdom of Thailand with respect to dispatch of Japanese experts, Thai counterpart personnel training in Japan and provision of equipment by means of Technical Cooperation Forms A-1, A-2, A-3, and A-4, respectively.
(4) Other publications concerning the Project

2. Discussions and Observations

The Team discussed various aspects of the Project and observed the buildings, machinery, equipment, facilities and utilities made available for the Project.

To recognize the impact and efficiency of the training, discussions were held with counterparts trained in Japan.

Ⅲ. OBJECTIVE AND ACTIVITIES OF TECHNICAL COOPERATION FOR THE PROJECT

1. Objective of the Project

According to the Record of Discussions signed on July 31, 1990, the objective of the Project is to promote research necessary for control of infectious diseases prevailing in Thailand by introducing new technology, and by cooperating in the development of biological products.

2. Activities of Technical Cooperation

In order to accomplish the above-mentioned objective, both sides agreed that technical cooperation should be implemented for the following activities through dispatch of Japanese experts, acceptance of Thai counterpart personnel for technical training in Japan and provision of equipment.

Bacteriology

(1) To establish projects for epidemiological surveillance of diseases caused by bacteria such as diarrhea, pneumonia, legionnaire disease, melioidosis, nosocomial infection and enteric fever.

(2) To develop new techniques for diagnosis of bacterial diseases, and to simplify the existing ones and transfer them to Regional Medical Science Centers.

Virology

(1) To establish projects for epidemiological surveillance of viral diseases, i.e. DHF, JE, Polio, Rota, Hepatitis, RSV, HIV and

Rabies

(2) To cooperate in clinical trial of vaccines against some of the diseases indicated in (1)

(3) To develop new diagnosis techniques, simplify the existing ones and transfer them to Regional Medical Science Centers

(4) To study medicinal plants for anti HIV

Biological Products

(1) To continue the development of vaccines, i.e. pertussis component vaccine, rubella vaccine and purify chick embryo cell culture rabies vaccine

(2) To do the research development of fermentation technology for vaccine production

(3) To cooperate in clinical trials of thermostable DPT vaccine

(4) To strengthen the advanced techniques for biological control laboratory

Medical Entomology

(1) To focus on integrated vector control research and on the establishment of an insect vector surveillance system

(2) To promote insect reference museum activities

(3) To study the vector capacity of medically important insects

(4) To detect allergens in insects and mites

(5) To study the immunology of blood source of insect vector

Other Related Fields

(1) To study molecular biology and immunology for better understanding of the etiology and epidemiology of microbial diseases

(2) To introduce modern technology such as genetic engineering for better control of infectious diseases of public health importance through vaccine and reagent development

(3) To develop biological assays for detection of carcinogens and mutagens

(4) To study the effects of food contaminants on health with special reference to microbial toxins

(5) To strengthen the activities of common facilities, i.e. microbial monitoring of experimental animals and electron

microscopical technology

Ⅳ. PERFORMANCE OF THE PROJECT

1. Facilities

Construction of institute facilities and installation of equipment directly related to the activities of the Project were completed at the end of October, 1986 under the Japanese Grant Aid Programme. Other facilities (including electricity, gas; water supply systems, sewage system, telephone and furniture) necessary for implementation of the Project were provided by the Thai side.

The efforts made by the Government of the Kingdom of Thailand for provision of equipment, offices, laboratory, etc. are highly appreciated.

2. STAFFING

At present, a total of eighteen (18) Thai counterpart personnel have been assigned to the Project for effective implementation and successful transfer of technology. The list of the Thai counterpart personnel is presented in ANNEX 1.

3. MANAGEMENT AND ADMINISTRATION

All administrative and managerial services are being provided by the Thai counterpart personnel.

In the past, the Coordinating Committee of the following composition, was convened in the fiscal year 1990/1991 for smooth implementaton of the Project.

1) Chairman: Permanent Secretary, Ministry of Public Health
2) Thai side:
   a. Director-General, DMS
   b. Deputy Director-General, DMS (Director of NIH)
   c. Deputy Directors, NIH
   d. Principal medical officials, Ministry of Public Health
   e. A representative of the University Affairs Office
   f. A representative of the Department of Technical and Economic Cooperation

3) Japanese side:

a. Team Leader

b. Coordinator/Liaison officer

c. Other experts and personnel concerned dispatched by JICA

d. Assistant resident representative of JICA Thailand Office

4. JAPANESE EXPERTS

JICA has dispatched three (3) long-term experts and thirty-five (35) short-term experts whose names and fields are listed in ANNEX 2.

5. THAI COUNTERPART PERSONNEL TRAINING IN JAPAN

Thus far, six (6) Thai counterpart personnel have been sent to Japan for either observation or technical training, and three (3) more counterpart personnel are to be accepted in Japan in March 1992. Their names are listed in ANNEX 3.

JICA accepted the Thai counterpart personnel in the fields agreed in the Record of Discussions. Their technical training was very effective for obtaining useful information.

6. EQUIPMENT

Between 1990 and 1992, equipment worth about 45 million yen was donated by the Government of Japan. The main equipment items and machinery are listed in ANNEX 4.

Equipment for the Project provided by the Government of Japan has been used efficiently in the activities of the Project.

7. BUDGET

A summary of the Project cost spent by Japanese and Thai sides is shown in ANNEX 5. Both sides made the best effort to secure the budget necessary for implementation of the Project.

8. ACCOMPLISHMENT OF TECHNICAL COOPERATION

Through careful evaluation and discussions, the both sides agreed that the technical cooperation implemented during the extension period has accomplished the afore-mentioned objectives.

Especially, a big progress is seen in various fields of the research and reference activities, such as;

- the molecular studies in virology and bacteriology
- the wider application of HIV diagnostic kits, which had been established in the first stage of the Project, supplying them to outside institutes at request
- diagnostic microbiology of enteric bacteria, including enteropathogenic E.coli, Salmonella, and Clostridium difficile
- technology transfer for the production of tissue culture-derived rabies vaccine was completed, and bulk preparations of an enough amount of field trial has already been pooled

The progress is referred in detail in "Summary of Activities, Research Promotion Project, National Institute of Health (NIH), Department of Medical Sciences, August 1990 to January 1992".

It is pointed out that the common facilities equipped by the Project such as;

(1) Laboratory Animal Center
(2) Biohazard Laboratory
(3) RI Laboratory
(4) Equipment Center

have been maintained in good conditions enough to extend services to laboratory activities in NIH.

It is also mentioned that many Thai counterpart personnel were stimulated and encouraged in their science activities. Many of them proceeded to higher trainings such as master courses in the universities and some others are now under Ph.D. course in Japan.

On the other hand, the objective aimed in the extension period was not fulfilled in some aspects in spite of the efforts of both sides.

It is suggested that the following fields, for instance, still should be supported for full accomplishment.

- the development of diagnostic measures for some infectious diseases

- the wider use of diagnostic kits which have been developed in the past years of the Project for the purpose of epidemiology

- the further strengthening of human resources for achieving both research and diagnostic works in NIH.

IV. CONCLUSION

As the results of the joint evaluation and discussions, both sides reached the following conclusions:

1. In general, the goals of most activities of the Project, as stipulated in the Record of Discussions concerning Extension of the Period of Technical Cooperation are now beginning to be realized.

2. It is, however, pointed out that the objective aimed in the extension period was not fulfilled in some aspects and further support still be needed.

3. It is advisable to strengthen the role of NIH as the national reference center for infectious diseases including AIDS and its related infectious diseases.

4. "The third country training program" will be recommended to start in not so distant future with the function of a South-East Asia Reference Regional Center.

Remark: It is advisable to apply modern technology obtained in this Project to other fields of medical sciences.

ANNEX I

LIST OF THAI COUNTERPART PERSONNEL
(STAFF OF EACH DEPARTMENT)

DEPARTMENT	NAME	POSITION
1. Bacteriology	Miss Krongkaew Supawat	Scientist
	Mrs. Wimol Petkanchanapong	-ditto-
	Mrs. Pimjai Naigowit	-ditto-
	Mrs. Aroon Bangtrakulnonth	-ditto-
	Mrs. Surang Dejsirilert	-ditto-
2. Virology	Miss Sirima Pattamadilok	Scientist
	Mrs. Kasama Supanaranond	-ditto-
	Dr. Yaowapa Pongsuwanna	-ditto-
	Miss Suntharee Rojanasuphot	-ditto-
	Dr. Pornthip Samuthananon	-ditto-
3. Biological products	Mr. Prakorb Ruangrairatanaroj	Scientist
	Mr. Prayuth Buddirakkul	-ditto-
4. Medical entomology	Mr. Chitti Changsaeng	Scientist
	Mrs. Usavadee Thavara	-ditto-
5. Research on Health Sciences	Mr. Wattana Auwanit	Scientist
	Dr. Tanawat Nantamingcharoen	-ditto-
	Mrs. Duangthanorm Tavaranantha	-ditto-
	Mr. Mongkol Chenchittikul	-ditto-

ANNEX 2

LIST OF JAPANESE EXPERTS DISPATCHED BY JICA

NO.	JAPANESE FISCAL YEAR	NAME	PERIOD	FIELD
(LONG TERM EXPERT)				
1.	1985 ～1992	Mr. Kohei Nakajima	85. 8. 1～92. 7.31	Coordinator
2.	1987 ～1992	Dr. Komi Kanai	87. 6.15～92. 7.31	Project Leader
3.	1990 ～1992	Dr. Eiko Kondo	90.11. 1～92. 7.31	Bacteriology
(SHORT TERM EXPERT)				
1.	1990 ～1991	Dr. Masaaki Tsuno	90. 8.15～90.12.14	Bacteriology
2.		Dr. Kuniaki Sakamoto	90. 8.21～90.12.28	Rabies Vaccine
3.		Dr. Saburo Iwasa	90.11. 5～91. 4. 4	Quality Control of Acelluar P. vaccine
4.		Dr. Motoyoshi Mogi	90.11. 7～91. 1.31	Medical Entomology
5.		Dr. Takeshi Kurata	90.11.24～91.12.15	Histopathology
6.		Dr. Yutaka Tatebe	90.12. 3～90.12.28	Molecular Immunology
7.		Dr. Shudo Yamazaki	90.12.10～90.12.16	Virology
8.		Dr. Haruo Watanabe	90.12.10～90.12.16	Bacteriology
9.		Dr. Shigehar Ueda	90.12.10～90.12.16	Vaccine
10.		Ms. Michiyo Hashiguchi	90.12.10～90.12.16	Cooperation Planning
(NO.7-10 Consultation Expert Team)				
11.		Dr. Naokazu Takeda	90.12.10～90.12.31	Molecular Study of Enterovirus Group
12.		Dr. Kunihiro Nerome	90.12.20～91. 1.17	Molecular Epidemiology of Influenza
13.		Dr. Hirofumi Danbara	90.12.25～90. 1.24	Molecular Epidemiology
14		Dr. Koki Taniguchi	90.12.26～91. 1 15	Molecular Study of Rotavirus
15.		Mr. Kazuo Goto	91. 1.19～91. 3. 2	Experimental Animals

16.	Dr. Akira Igarashi	91. 2.13～91. 3. 2	Molecular Study of JE and Dengue Virus
17.	Dr. Koichi Morita	91. 2.28～91. 3.28	Molecular Study of JE and Dengue Virus
18. 1991 ～ 1992	Dr. Yoshito Wada	91. 5. 9～91. 6. 5	Ecology Study for Japanese Encephalitis
19	Dr. Sachio Tokiyoshi	91. 6. 2～91. 7.16	Rabies Virus
20	Dr. Koichi Morita	91. 8. 6～91. 9. 3	Molecular Study of JE and Dengue Virus
21	Dr. Osamu Nishio	91.11.14～91.12.14	Isolation and Identification of Polio Virus and Determination of the Neutralizing Antibodies
22	Dr. Kuniaki Nerome	91.11.16～91.12. 5	Molecular Epidemiology
23	Dr. Takeshi Kurata	91.12.17～92. 1. 6	Histopathological Diagnosis of Infectious Diseases by Immunocytochemical Methods
24	Dr. Kazuyoshi Ikuta	91.12.17～92. 1. 6	Biotechnology
25	Dr. Takayuki Ezaki	91.12.26～92. 1.10	Development of Diagnostic DNA Probe Toxigenic Psuedomonas Pseudomallei
26	Dr. Yumiko Furuya	92. 1. 6～92. 2. 5	Richettsia Diseases
27.	Dr. Ryosuke Murata	92. 1.16-92. 1.22	Bacteriology
28	Dr. Akira Oya	92. 1.16-92. 1.22	Virology

29	Dr. Koichi Soga	92. 1.16-92. 1.22	Cooperation Planning
30	Mr. Toshimichi Aoki	92. 1.16-92. 1.22	Technical Cooperation
	(NO. 27-30 Planning Survey Expert Team)		
31	Dr. Tohru Tokunaga	92. 2.26-92. 3. 4	Bacteriology
32	Dr. Michiaki Takahashi	92. 2.26-92. 3. 4	Virology
33	Dr. Koichi Yamanishi	92. 2.26-92. 3. 4	Immunology
34	Mr. Toshimichi Aoki	92. 2.27-92. 3. 4	Planning Evaluation
35	Mr. Hisakatsu Okuda	92. 2.26-92. 3. 4	Cooperation Planning
	(NO. 31-35 Evaluation Expert Team)		

ANNEX 3

LIST OF COUNTERPART PERSONNEL SENT TO JAPAN

JAPANESE FISCAL YEAR	NAME	TRAINING PERIOD	TRAINING FIELD
1990-1991	Mrs. Kanchana Leelasiri	90. 8.28-90.11.28	Vaccine Development
	Mrs. Raywadee Butraporn	90. 9.20-91. 8.27	Planning of Microbiological Monitoring System
	Miss Naiyana Kanogsunchanrat	90.10. 7-91. 8. 7	Maintenance of of Analytical Instruments
	Mr. Paijit Waranchit	91. 2.21-91. 5. 1	Biotechnology
1991-1992	Dr. Jakkriss Bhumisawasdi	91.11.12-92. 1.28	Research on Control of Infectious Disease
	Mrs. Mayura Kusum	91.11.12-92. 1.28	Research on Control of Infectious Disease
	Mrs. Wilai Chalermchan	92. 3.24-93. 3.16 (Plan)	Bacterial Immunology
	Miss Sumlee Pothipunya	92. 3.24-93. 3.16 (Plan)	Molecular Biology
	Dr. Chongdee Wongpinairat	92. 3.23-92. 4. 5 (Plan)	Management of Health Laboratories

ANNEX 4

PROVISION OF EQUIPMENT

JAPANESE FISCAL YEAR	ITEMS OF MAIN EQUIPMENT	AMOUNT (YEN)
1990-1991	High Pressure Steam Sterilizer	¥25,000,000
	Concentrator	
	Polymerase Chain Reaction Machine	
	Autoclave	
	Microfilm Reader Printer	
1991-1992	Fermenter	¥20,000,000
	DNA Synthesizer	
	Stainless Steel Cover of Mouse Cage	
	DNA Photographic System	
	2-Way Valve for Fan Coil Unit	
	Gas Chromatograph Accessory	
	Airborne Dust and Microorganism Collector	
	NI Resistance Bulb(Air Handling Unit)	
	Combination PH Electrode	
	Vinyl Isolator	
	Rat Cage	
	Blower for Animal Isolator	
	16mm Planetary Microfilmer	
	Spare parts for Connecting HPLC and Computer	
	Accessories for make up of computers	

ANNEX 5

SUMMARY OF PROJECT COST

(Unit :Thousand Yen)

JAPANESE FISCAL YEAR	1990-1991	1991-1992	TOTAL
COST OF DISPATCH OF EXPERTS	83,474	58,233	141,707
COST OF PROVISION OF EQUIPMENT	25,000	20,000	45,000
COST OF DISPATCH OF SURVEY TEAMS	0	0	0
COST FOR MIDDLE LEVEL STAFF TRAINING	5,209	0	5,209
OTHER LOCAL RUNNING COST	0	0	0
OTHERS	693	461	1,154
TOTAL	114,376	78,694	193,070

Source of NIH Fund

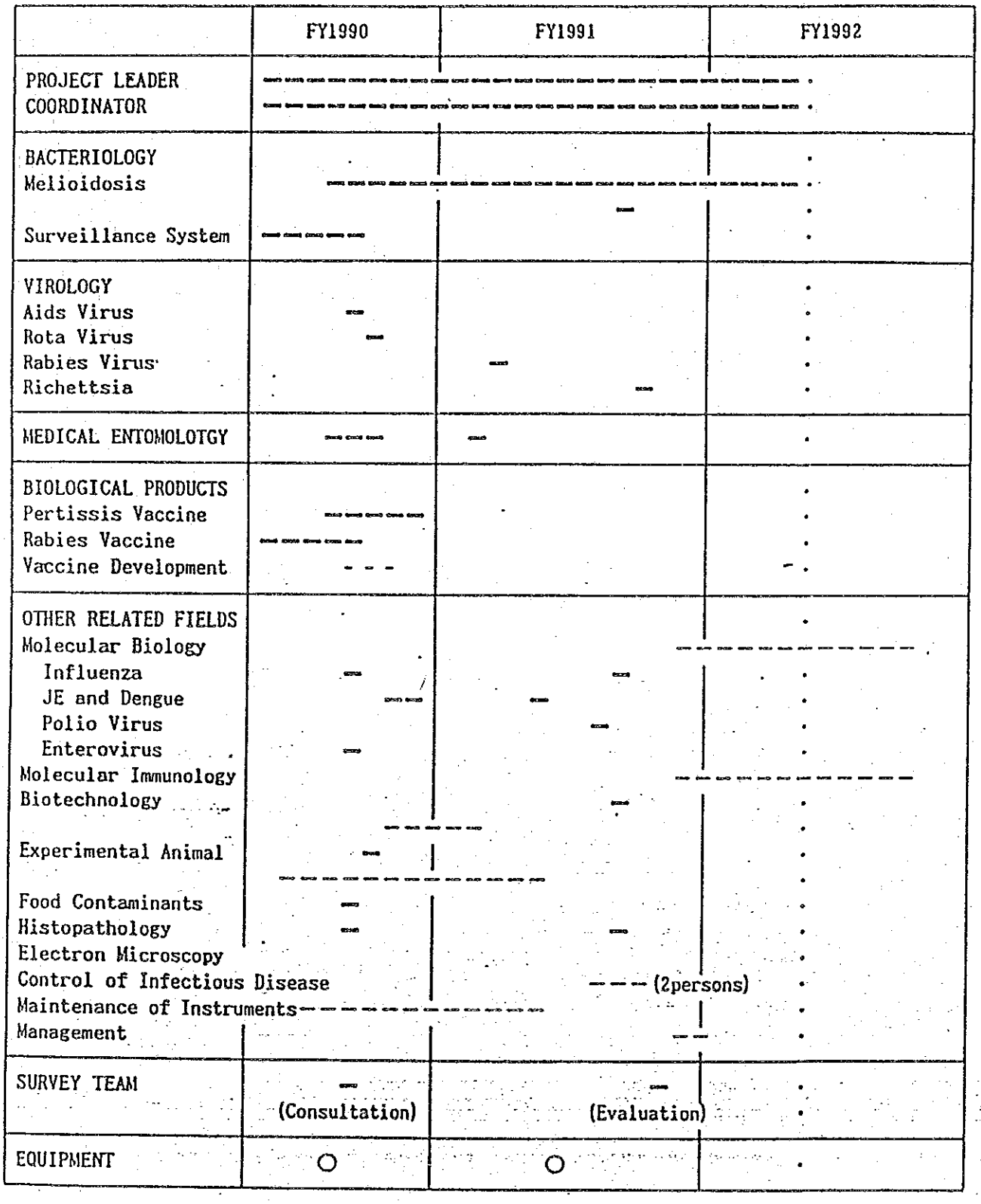
(Unit:Baht)

THAI FISCAL YEAR	1990-1991	1991-1992	TOTAL
GOVERNMENT BUDGET	42,787,082	51,719,000	94,506,082
OTHER	2,655,632	3,212,618	5,868,250
FOREIGN AIDS	1,580,302	1,697,477	3,277,779
TOTAL	47,023,016	56,629,095	103,652,111

Note: This table is as of February, 1992
Japanese fiscal year is from April 1 to March 31.
Cost of training of counterpart personnel is not included in this table.

ANNEX 6

ANNUAL SCHEDULE FOR NATIONAL INSTITUTE OF HEALTH PROJECT



—— Dispatch of experts
- - - Counterpart Training in Japan

② Steering Committee資料

(1) Summary of Project Activities 1985-1992

Steering Committee Meeting

(40-2/1992)

Friday 28 February 1992

9:30 A.M., Room A-203

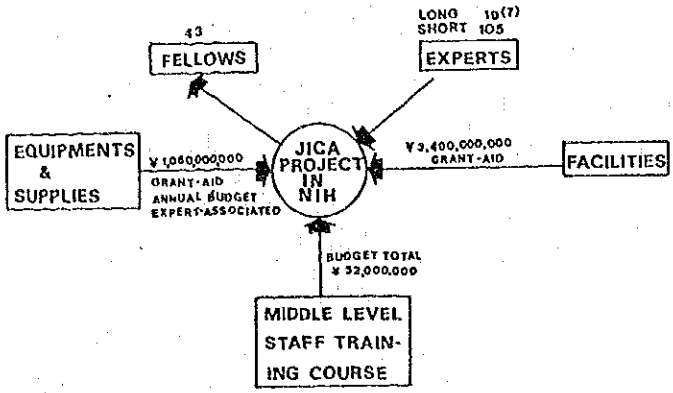
National Institute of Health

Agenda

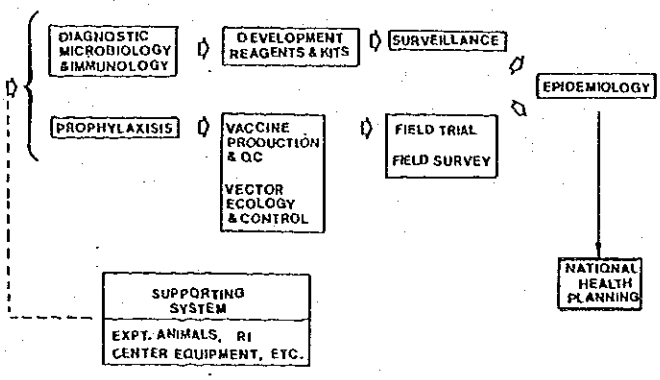
1. Information from Chairman and Japanese Project Leader
2. Brief Summary of the Past Activity of the Project (Attachment 1)
3. Report on Input by JICA for Extension Period (Attachment 2-6)
4. Achievement of the Project Activities 1990-1991 FY Fiscal Years (Attachment 7)
5. Work Plan (Attachment 7)
6. Preliminary Study of Draft of Evaluation Report (Attachment 8)
7. Others (if any)

SUMMARY OF PROJECT ACTIVITIES
1985 – 1992

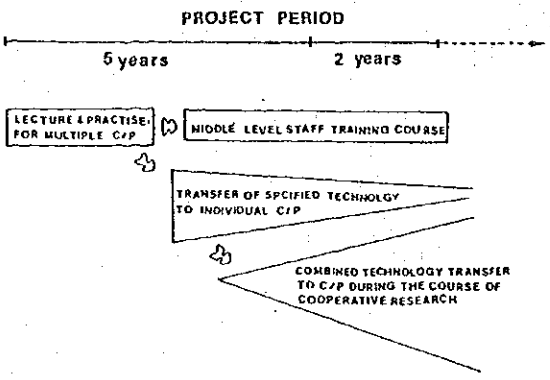
MECHNANISM OF COOPERATION



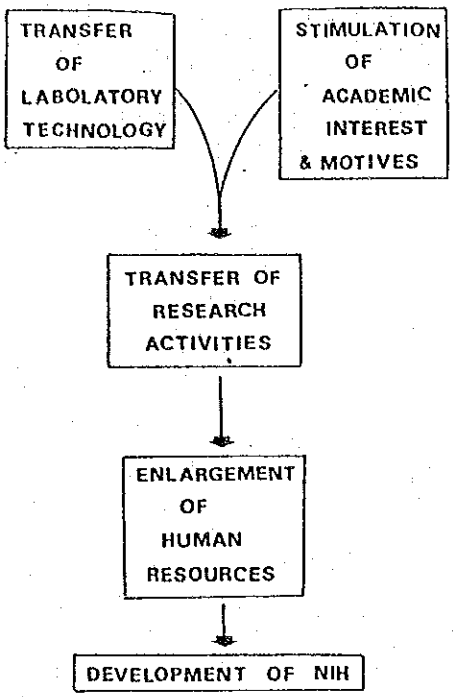
STRATEGY IN INFECTIOUS DISEASE CONTROL POJECT
BASIC IDEAS DEFINED IN "RD"



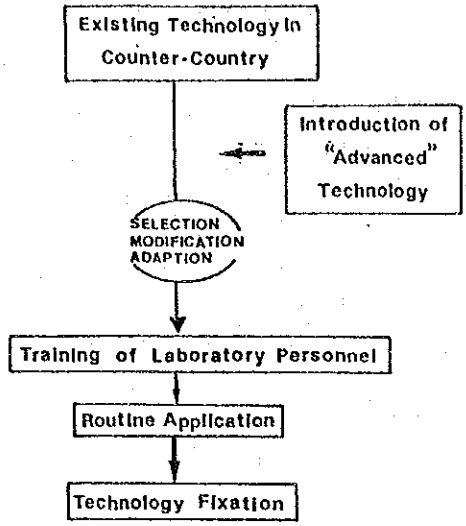
THE CHANGE IN THE STRATEGY OF TECHNOLOGICAL COOPERATION



RESEARCH PROMOTION PROJECT



TECHNOLOGY TRANSFER



WHAT INFECTIOUS DISEASES ARE TO BE TAKEN UP IN NIH PROJECT

THE GLOBAL OR INTERNATIONAL LEVEL

AIDS, Influenza,
WHO-Oriented diseases (vaccine-preventable)

THE DOMESTIC OR ENDEMIC LEVEL

Dengu, Japanese-encephalitis, Melioidosis

THE TROPICAL LEVEL GENERAL

Infectious diarrhoea, Malaria

OTHERS

Rickettsia diseases, Keptospirosis

Cooperative Research Activities

Molecular biology and epidemiology of virus infections

- HHV
- Rota
- Influenza
- HIV
- Dengu
- JE
- HCV
- AHCV (coxsackievirus A 24)

Molecular biology of Salmonella

Melioidosis research

Technology Transfer in NIH Projects (1)

Area	Transferred Technologies
Diagnostic bacteriology	General laboratory techniques
	Numerical classification
	Special identification method
	Fatty acid pattern (GLC)
	DNA hybridization
	Plasmid pattern
	Phage typing
	MIC test method
	Immunofluorescent microscopy
	Species identification method
	Mycoplasma
	Legionella
	Gram-negative nonfermentative bacteria
	Nocardia
	Rickettsia
	Toxin or antigen purification
	V. cholerae
	C. difficile

Technology Transfer in NIH Projects (2)

Area	Transferred Technologies
Diagnostic virology	Preparation of various antigen & antibodies
	Various serological tests
	Molecular techniques
	RNA-PAGE
	Cloning
	Sequence determination
	PCR
	Monoclonal antibodies
	Preparation of diagnostic kits (AIDS-IFA, etc)
Vaccine production and quality control	Japanese encephalitis (completed)
	Rabies (completed)
	Measles (not completed)
	Pertussis (not completed)

Technology Transfer in NIH Project (3)

Area	Transferred Technologies
Entomology	Museum
	Ecological of vector mosquitoes
	Control of vector mosquitoes
Laborstory animals	Maintenance of laboratory animals
	Microbiological monitering
	4 strains of SPF mouse colonies
	Guinea pig colony
	Hypertension rats
	SPF quails
Others	P3 laboratory
	RI

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2 Text: Bacteriological examination in clinical microbiology, by Dr. Sakazaki

3 Murata, R. (1981): Introduction to quality control of biological products.

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LIST OF EXPERT (FY 1990/91)

No.	Name	Field	Period	Counterpart	Equipment Brought By Expert
1.	Dr. Masaaki Tsuno	Taxonomy of gram-negative bacteria	August 15 - December 14, 1990	Miss Krongkaew Supawat	300,000 YEN FOB
2.	Dr. Kuniaki Sakamoto	Rabies Vaccine	August 21 - December 27, 1990	Mr. Prakorb · Ruangrairatanaroj	355,666 YEN CIF
3.	Dr. Saburo Iwasa	Quality control of acellular pertussis vaccine	November 5, 1990 - April 4, 1991	Mr. Prayuth Buddhirakkul Mrs. Wimol Petkanchanapong	372,100 YEN FOB 81,544 YEN CIF
4.	Dr. Eiko Kondo (Long term)	Diagnostic immunology of melioidosis and chemical taxonomy of pathogenic fungi	November 1, 1990 - July 31, 1992	Mrs. Pimjai Naigowit	372,050 YEN FOB 186,070 YEN FOB
5.	Dr. Motoyoshi Mogi	Mosquito ecology	November 7, 1990 January 31, 1991	Mr. Chitti Chansaeng	719,530 YEN FOB
6.	Dr. Takeshi Kurata	Histo pathology	November 23 - December 14, 1990	Mrs. Pimjai Naigowit	384,605 YEN FOB 593,260 YEN FOB
7.	Dr. Kuniaki Nerome	Molecular epidemiology of influenza	December 20, 1990 January 17, 1991	Miss Sirima Pattamadilok	1,418,886 YEN FOB
8.	Dr. Yutaka Takebe	Molecular immunology	December 3-28, 1990	Mr. Wattana Auwanit	265,740 YEN FOB
9.	Dr. Naokazu Takeda	Molecular study on enterovirus group	December 10-30, 1990	Mrs. Kasama Supanaranond	433,799 YEN FOB 227,000 YEN FOB
10.	Dr. Shudo Yamazaki	Mission	December 10-16, 1990		203,720 YEN FOB
11.	Dr. Shigeharu Ueda	Mission	December 10-16, 1990		
12.	Dr. Haruo Watanabe	Mission	December 10-16, 1990		
13.	Mrs. Michiyo Hashiguchi	Mission	December 10-16, 1990		
14.	Dr. Hirofumi Danbara	Molecular epidemiology of bacteria	December 25, 1990 January 24, 1991	Mrs. Aroon Bangtrakulnonth	
15.	Dr. Koki Taniguchi	Rotavirus	December 26, 1990 January 15, 1991	Dr. Yaowapa Pongsuwanna	
16.	Dr. Kazuo Goto	Microbiological monitoring of animal	January 19 - March 2, 1991	Dr. Tanawat Nantamingcharoen	
17.	Prof. Akira Igarashi	JE and dengue virus	February 13 - March 1, 1991	Miss Suntharee Rojanasuphot	

18. Dr. Kouichi Morita	JE and Dengue virus	February 28 - March 27, 1991	Miss Suntharee Rojanasuphot	430,746 YEN FOB
Dr. Komi Kanai				199,610 YEN FOB 2,172,030 YEN CIF

LIST OF EXPERT (FY 1991/92)

No.	Name	Field	Period	Counterpart	Equipment Brought By Expert
1.	Prof. Yoshito Wada	Ecology study for Japanese encephalitis epidemiology	May 9 - June 5, 1991	Mrs. Usavadee Thavara	505,000 YEN FOB
2.	Dr. Yukio Tokiyoshi	Rabies virus	June 2 - July 16, 1991	Dr. Pornthip Samuthananon	324,000 YEN FOB
3.	Dr. Kouichi Morita	Molecular study of JE and dengue viruses	August 6 - September 2, 1991	Miss Suntharee Rojanasuphot	765,000 YEN FOB
4.	Dr. Osamu Nishio	Isolation and identification of polio virus and determination of neutrilizing antibodies	November 14 - December 14, 1991	Dr. Yaowapa Pongsuwanna Mrs. Kasama Supanaranond	54,050 YEN FOB 642,728 YEN CIF 553,000 YEN CIF
5.	Dr. Kuniaki Nerome	Molecular epidemiology	November 16 - December 5, 1991	Miss Sirima Pattamadilok	300,000 YEN FOB
6.	Prof. Kazuyoshi Ikuta	Molecular biology of biotechnology	December 17, 1991 January 5, 1992	Mrs. Duanthanorm Tavaranantha	397,560 YEF FOB
7.	Dr. Takeshi Kurata	Histopathological diagnosis of infectious diseases by immunocytochemical methods	December 17, 1991 January 6, 1992	Mrs. Pimjai Naigowit	609,172 YEN FOB
8.	Prof. Takayuki Ezaki	Development of diagnostic DNA probe toxigenic pseudomonas pseudomallei	December 26, 1991 January 10, 1992	Mrs. Surang Dejsirilert	200,200 YEN FOB
9.	Dr. Yumiko Furuya	Rickettsia diseases	January 6, 1992 February 5, 1992	Mr. Mongkol Chenchittikul	333,840 YEN CIF
Dr. Komi Kanai, Dr. Eiko Kondo					551,000 YEN CIF
Dr. Eiko Kondo					352,000 YEN CIF

LIST OF FELLOWSHIP (FY1990/91, FY1991/92)

No.	Name	Field	Period
(FY 1990/91)			
1.	Mrs. Raewadee Butraporn	Microbiological monitoring of experimental animals	September 19, 1990 - September 19, 1991
2.	Mrs. Kanchana Leelasiri	Administration of vaccine production and Q.C.	August 28, 1990- November 28, 1990
3.	Ms. Naiyana Kanogsunchanrat	Maintenance of analytical equipments	October 5, 1990- August 8, 1991
4.	Dr. Paijit Warachit	Virology in general	February 20, 1991- May 3, 1991
(FY 1991/92)			
1.	Dr. Jakkriss Bhumixawasdi	Biotechnology molecular - Biology (Gene cloning, DNA hybridization) RI Immunology	November 12, 1991- January 26, 1992
2.	Dr. Mayura Kusum	Administration General clinical pathology - Melioidosis - Fungi - Parasite - Nosocomial infection	November 12, 1991 - January 28, 1992
3.	Ms. Wilai Chaloemchan	Bacterial immunology	March 26, 1992 - March 23, 1993
4.	Ms. Sumlee Pothipunya	Molecular virology (JE)	March 24, 1992- March 23, 1993
5.	Dr. Chongdee Wongpinairat	Administration	March 23 - April 6, 1992

Provision Of Equipment (FY 1990/91, 1991/92)

No.	Item	Price (Baht)	Delivery Date	Division
(FY 1990/91)				
1.	High Pressure Steam Sterilizer	1,916,230	March 15, 1991	Health Sciences Research Institute
2.	Duplicator (Xeroprinter)	300,000	January 18, 1991	General Office
3.	Computerized ELISA Plate Reader	411,480	February 27, 1991	Health Sciences Research Institute
4.	Concentrator (For HIV)	365,000	December 7, 1991	Health Sciences Research Institute
5.	Polymerase Chain Reaction Machine	290,000	February 25, 1991	Health Sciences Research Institute
6.	Microcentrifuge	195,000	February 27, 1991	Health Sciences Research Institute
7.	Autoclave	90,720	February 27, 1991	Health Sciences Research Institute
8.	Cooler System, Handy Aspirator	72,850	February 27, 1991	Health Sciences Research Institute
9.	Microfilm Reader Printer	446,400	December 14, 1991	Health Sciences Research Institute
	Total	4,087,680		
(FY 1991/92)				
1.	Fermenter	960,000		Biological Products
2.	DNA Synthesizer	640,000		Health Sciences Research Institute
3.	Stainless Steel Cover of Mouse Cage	206,250	January 10, 1992	Health Sciences Research Institute

No.	Item	Price (Baht)	Delivery Date	Division
4.	DNA Photographic System	120,000		Clinical Pathology
5.	2-Way Valve for FCU (NC)	41,400	December 26, 1991	Health Sciences Research Institute
6.	Accessory of Gas Chromatograph	84,960	December 6, 1991	Health Sciences Research Institute
7.	Airborne Dust and Micro-organism	139,300		Toxicology
	Collector			
8.	NI Resistance Bulb	39,060	December 26, 1991	Health Sciences Research Institute
9.	Combination pH Electrode	26,400	December 9, 1991	Biological Products
10.	Vinyl Isolator	238,200	January 10, 1992	Health Sciences Research Institute
11.	Rat Cage	748,500	January 10, 1992	Health Sciences Research Institute
12.	Blower for Animal Isolator	136,000	January 10, 1992	Health Sciences Research Institute
13.	16 mm. Planetary Micro filmer	112,500	October 16, 1991	Health Sciences Research Institute
14.	Sparepart for Connecting HPLC	48,000		Clinical Pathology
	and Computer			
15.	Accessories for make up Computer	90,000	September 23, 1991	Medical Entomology
	Total	3,630,570		

Training Courses for Middle Level Staff (1990/91)

No.	Course	Budget (Baht)
1.	QCC	455,000
2.	Research Methodology	100,000
3.	Application of Biotechnology	100,000
4.	Phage Typing Technique of Staphylococcus aureus	40,000
5.	Training Courses in Public Health Promotion	
5.1)	Clinical Microbiology	50,000
5.2)	Food Sampling Technique for Microanalysis Qualitation	12,000
5.3)	Transfer of Appropriate Technology of Diagnosis Shigellosis	60,000
5.4)	Workshop on Working Cooperation and Cooridation	78,000
	Total	895,000

Annual Cooperation By JICA

Fiscal Year		1985	1986	1987	1988	1989	1990	1991	Total	1992 (Proposal)
1. Expert: (Person)	Long Term	2	1	2			1		6	
	Short Term	8	12	22	21	15	17	9	104	9
2. Fellowship (Person)		7	7	6	7	4	4	5	40	5
3. Equipment: (Ten Thousand Baht) (One Million Yen)	Annual Provision	270 (22)	347 (23)	813 (45)	1143 (60)	1429 (80)	408 (25)	363 (19)	4773 (274)	322
	Carried By Expert	114 (9)	461 (30)	701 (38)	473 (24)	130 (7)	200 (12)	43 (2)	2122 (122)	
4. Training: (Ten Thousand Baht)	Middle Level Staff	--	19	190	170	129	89	--	597	
	Annual Seminar	--	--	--	8	9	10	11	38	11
5. Another Local Activity (Ten Thousand Baht)		30	23	28	40	12	19	15	167	25
Exchange Rate: 1Baht= Yen		8.1	6.6	5.5	5.2	5.5	6.1	5.2		5.1

(3) Summary of Activities (August 1990-January 1992)

SUMMARY OF ACTIVITIES

RESEARCH PROMOTION PROJECT

NATIONAL INSTITUTE OF HEALTH (NIH)

DEPARTMENT OF MEDICAL SCIENCES

August 1990 to January 1992

Prepared for the Japanese Final Evaluation Team

JAPAN INTERNATIONAL COOPERATION AGENCY

Visiting the NIH, Thailand, in February 1992

TABLE OF CONTENTS

SUMMARY OF ACTIVITIES DURING THE EXTENSION PERIOD

1. RESEARCH DEVELOPMENT

1.1 The Complete Nucleotide Sequence of a Variant of Coxsackievirus A24

A variant of Coxsackievirus A24 (CA24v) is one of the agents causing of acute hemorrhagic conjunctivitis (AHC). Since CA24v emerged as a new human pathogen from a single focus, like enterovirus 70 (EV70), the virus provides us a unique example to find a clue to indentify the origin of the virus as well as to know the evolutionary relationship among other picornaviruses, we have determined the complete nucleotide sequence of the standard strain of CA24v, the EH24/70 strain.

The genome of CA24v is 7,461 nucleotides long with poly (A) tail at 3' end. Following a 750 nucleotides 5' non-coding region, there is long open reading frame of 7,392 nucleotides, which serve to encode a viral polyprotein consisting of 2,214 amino acids. Comparison of the deduced amino acid sequence the polyprotein with that of known enteroviruses allowed us to predict the possible cleavage sites.

The overall structure and the organization of the RNA genome is typical for an enterovirus. Based on the similarity of the nucleotide sequence of the 5' and 3' non-coding regions together with the amino acid sequence of the encoded proteins, EH24/70 appeared to be closely related to poliovirus and coxsackievirus A21.

1.2 Purified Toxins of Clostridium difficile and Related Organisms

Toxins from the causative agents of diarrheal diseases, Clostridium difficile, C. sporogenes and Klebsiella oxytoca have been purified by using ammonium sulfate precipitation and DEAE-sepharose column chromatography. Four major peaks were obtained from both C. difficile A 4897 standard strain and CD11 isolated strain. K. oxytoca also gave four major peaks with different pattern form C. difficile. For C. sporogenes, only two major peaks have been shown. Some major peaks from C. difficile have strong cytotoxic activities, but the other peaks have shown haemagglutinated properties. Anti-toxin production from those purified toxins would be done for diagnostic test in diarrheal specimen.

1.3 Effects of Four Antibiotics on Adherence and Haemagglutinating Properties of Enteroaggregative E. coli

The effect of four quinolones drugs (tosufloxacin, ofloxacin, norfloxacin and ciprofloxacin) to E. coli that could adhere to human intestine was studied. All four drugs caused cell elongation, decreased cell mortality and interfered fimbriae formation. According to the adherence inhibilition level, the most effective drug was tosufloxacin, ciprofloxacin, ofloxacin and norfloxacin, respectively.

1.4 Study on Molecular Genetics of Enteroaggregative E. coli

The DNA fragment(s) encoding haemagglutinin gene(s) of the new diarrheagenic E. coli group (strain TL-100) was determined. Two plasmids (71.5 MDa and 31.2 MDa) were found. Six HA non-producing mutants which had no. 71.5 MDa plasmid were selected from 25 conjugants. However, transformants containing big plasmid that showed haemagglutinating activity were also obtained.

1.5 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

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.

The Government of Thailand has imposed a policy on expanding international markets of agricultural products especially food products. A great variety of food has been produced for domestic consumption and for export. Quality and safety assessment of these products are essential in building up popularity.

The project aims at technical improvement and strengthen the capabilities of Salmonella identification in food control laboratories in order to expand services in the quality promotion of food for export.

To compare the sensitivities, the expense and time consumption of the colorimetric DNA hybridization technique using Gene-Trak Salmonella Assay Kit (Catalog Number GT 0605) and

conventional cultural procedure for identification of the Salmonella in frozen food products.

The results showed that total of 70 frozen chicken meat samples and 20 frozen seafood samples were examined for both methods. It took 3 days using colorimetric DNA hybridization method and 5 days using conventional cultural method (culture, biological test and serotype). It was found that Salmonella were detected from 2 samples (2.22%) using colorimetric DNA hybridization method and 19 samples (21.22%) by conventional cultural method. The value of the positive control of Gene-Trak Salmonella Assay Kit is lower than the value of positive control stated in the kit manual. Kit test costed 506 baht/sample whereas conventional cultural method costed only 33 baht/sample.

In conclusion, the comparative study showed that the Gene-Trak Salmonella Assay kit was not comparable to the conventional cultural method in Salmonella detection in food. It could be possible that the efficiency of the test decreased with time and caused inferiority of the kit test. It might be any other causes. Confirmation test could not be done because of the limitation of the kit test.

1.6 Adherence Sites of Aeromonas hydrophila in Human or Animal Intestines

By using formalin-fixed human or animal intestinal mucosa, we investigated the adherence sites of clinical isolated Aeromonas hydrophila. A. hydrophila strain produced cell hemag

glutinins (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 surface. 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 comparble 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.

1.7 Bacterial Chemotaxonomy

Under the supervision of Dr. Ezaki, DNA-DNA hybridization technique for bacterial identification was transferred. Research or development of identification by DNA hybridization are underway in identification of Acinetobaeter into genospecies level. Measurement of guanine-cytosine content of bacterial DNA by reverse-phase high-performance liquid chromatography are new going on, under the consultation of Dr. Ezaki.

2. DEVELOPMENT AND IMPROVEMENT OF DIAGNOSTIC PRODUCTS AND TECHNIQUES

2.1 Molecular Diagnosis of Dengue viruses using RT-PCR Technique

Diagnosis of dengue viruses using RT-PCR was developed in Arbovirus unit NIH, DMS. The technique was tested with Rayong samples, in comparison with virus isolation method. From twenty eight positive virus isolated samples, there were twenty two samples which were completely coincided by RT-PCR. Furthermore, the techique was rapid enough to detect and identify the viruses within two-day test, and various forms of samples could be analysed with or without sample extraction. Some details of the technique might be further studied before using it as diagnostic method for dengue virus infection in human.

2.2 Preparation of Dengue and JE Antigens for Antibody Capture ELISA

The optimal conditions for dengue and JE virus antigens from tissue culture were innovated. Twelve strains of dengue viruses serotypes 1 to 4 and two strains of JE viruses from different sources were cultured in C6/36 cells. Firstly, a more versatile mosquitoes cell line was replaced the former one. Secondly, various virus seeds were studied to find out the proper sources. Finally, the antigens from tissue culture were daily checked for HA unit and ELISA unit for ten days interval in order to find out the maximal dates for each viruses. The optimal HA titers of these antigens were at 6-10 days, 2-4 days and 4-10

days post infection for dengue serotypes 1 and 2, serotypes 3 and 4 and JE viruses, respectively. The optimal ELISA titers were at 10 days, 4-10 days, 4 days, 2-4 days and 4 days post infection for dengue serotypes 1,2,3,4 and JE viruses, respectively.

2.3 Enzyme Immuno Assay for the Detection of Rabies Neutralizing Antibody

An EIA technique for detection of rabies neutralizing antibody has been developed. Forty immunized sera were studied by using this technique. The results compared to those of the standard mouse neutralization test, show no statistical significant difference.

It has proved that, EIA is a specific, convenient and useful method for the quantitative assay of rabies virus neutralizing antibody.

2.4 An IgM ELISA for the Serodiagnosis of Poliovirus Infection

An IgM ELISA was developed to detect poliomyelitis IgM antibody for the diagnosis of poliovirus infection. The method is more rapid and simpler than the current tissue culture technique, and has no cross reaction with other enteroviruses. When we compared the positive rate between the microneutralization test, the serologic test of choice, and the IgM ELISA test, the latter was found to be more sensitive. From 49 suspected poliomyelitis cases, the positive rate of microneutralization test was 40.8% and IgM ELISA test was 67.3%

2.5 Application of Immunofluorescent Microscopy to Rapid Diagnosis of Melioidosis

Melioidosis caused by Pseudomonas pseudomallei is a serious infectious disease whose endemic areas are present in all the provinces of Thailand. The motality rate was as high as 83.7% in the septicemic cases. The morbidity rate is increasing every year. Bacause of the necessity of rapid diagnosis for the treatment of this disease, an attempt was made to develop indirect immunofluorescent microscopy with LPS or a protein fraction separated from P. pseudomallei as antigens. The antisera to these antigens were prepared using rabbits and guinea pigs. The specificity and sensitivity of our test system in the detection of the antigens was examined using 18 melioidosis specimens and 44 non-melioidosis specimens.

Fifteen (83.3%) out of 18 melioidosis samples diagnosed from clinical symtoms and culture-positive gave fluorescence-positive results. Six out of 44 non-melioidosis samples showed positive or unclear fluorescence.

Cross reaction in this immunofluorescent microscopy was examined between P. pseudomallei (ATCC type strain and clinical isolates) and other species (P. cepacia and E. coli). No cross reaction was observed when the anti-LPS guinea pig sera was employed, but the anti-LPS rabbit sera gave weak cross reaction.

From these results, we are encouraged to think that the immunofluorescent microscopy is useful as the rapid diagnostic method. The introduction of monoclonal antibody is now under consideration to improve the specificity of this method.

2.6 Immunofluorescent Microscopy for the Detection of IgM and IgG antibodies to Pseudomonas pseudomallei

Sera from 61 melioidosis patients, 37 other septicemic diseases and 80 healthy individuals were examined for the presence of IgM and IgG antibodies to Pseudomonas pseudomallei by indirect immunofluorescent antibody (IFA). From this study IFA-IgM titer > 1:8 and IgG > 1:64 were diagnostic value for melioidosis. The sensitivity, specificity and accuracy of IFA-IgM and IFA-IgG were 90.2%, 100.0%, 95.7% and 95.1%, 100.0%, 97.9% respectively.

The IFA test is rapid and reliable for the detection of P. pseudomallei antibody which may be value in differentiating acute and chronic melioidosis patients from other diseases.

2.7 Determination of Specific Antigen of Pseudomonas pseudomallei for the Diagnosis of Melioidosis

A certain protein from culture supernatant and outer membrane of P. pseudomallei was strongly immunogenic. Moreover, on the basis of indirect haemagglutination test it was found that sheep red blood cell sensitized with this protein react with higher titer against patient's antiserum than sheep red blood cell sensitized with lipopolysaccharide. As the results, this protein may play an important role in specific diagnosis of Melioidosis and pathogenesis of this organism.

2.8 Extraction of Bacterial Lipopolysaccharides from Pseudomonas pseudomallei for Immunodiagnosis of Melioidosis by Enzyme Linked Immunosorbent Assay

Lipopolysaccharide antigen is a major component of the outer membrane of Pseudomonas pseudomallei, which is extracted from heated cells by liquid phenol. With the lipopolysaccharide antigen prepared, a survey was made to measure the antibody level of 47 melioidosis sera, 55 non-melioidosis sera and 50 sera of healthy donors by enzyme linked immunosorbent assay (ELISA). The sensitivity, specificity and accuracy at ELISA cut off value > OD 320 were 95.7%, 94.2% and 94.7%, respectively.

The ELISA test is technically more rapid and reliable for the detection of antibody response to P. pseudomallei. It may be also of value in differentiating melioidosis from other infectious diseases.

2.9 The Production of Antigen and Antiserum for Serodiagnosis of Pulmonary Candidiasis

Candida albicaus serotype A CDC B 385 was used as an antigen to produce rabbit antiserum. The prepared antigen and antiserum reached the standard level of CDC product.

The efficacy and specification of our prepared antigen and antiserum were tested by using sera of 25 cases of pulmonary candidiasis patients, 15 cases of other bacterial infection patients, 5 cases of autoimmune diseases, 1 case of cryptococcosis and 20 cases of healthy blood donor. The results showed that 25 cases of pulmonary candidiasis revealed positive immunodiffus-

sion test, 21 cases gave identity band and 4 cases gave partial identity (cross reaction). Sera of other cases revealed negative immunodiffussion test. It is concluded that there is no false positive but there is cross reaction from other Candida spp. which could cause disease.

Presently, we provide the service on the diagnosis of systemic candidiasis by immunodiffussion test and also prepare the test reagents for Regional Medical Science Centers and other interested public health laboratories.

2.10 The Production of Fluorescein-Labeled and Antiglobulin for the Diagnosis of Aspergillosis by the Fluorescent Antibody Technique

Formerly, the FITC-labeled Aspergillus rabbit antisera produced and possessed many non specific reactions. Then the conjugation of new FITC with other lots of Aspergillus rabbit antisera had been carried out but it was unsuccessed. The pure culture smear of Aspergillus sp. could not revealed fluorescense of FICT after staining with direct fluorescent antibody technique.

We solved the problem by using the direct fluorescent antibody staining and immunoperoxidase staining for the diagnosis of Aspergillosis instead of direct fluorescent antibody technique. Aspergillus rabbit antisera (which had not been conjugated with FITC yet) was used as primary antibody for detecting Aspergillus antigen in clinical specimen, and then used the indirect

fluorescent antibody staining and immunoperoxidase staining. The result is very good.

2.11 Diagnosis of Mycotic Infection in Human Tissue Sections by Using the Immunoperoxidase Technique

We received the cooperation from the histopathologic unit of various hospitals in Bangkok in sending us the patients paraffin tissue sections which suspected candidiasis, Aspergillosis and histoplasmosis. Since in the sections, there were very low amount of hyphae and could not see the typical form of each fungus, we had to use the immunoperoxidase technique by using our prepared Candida albicans rabbit antiserum, our prepared Aspergillus rabbit antiserum, and commercial Histoplasma goat antiserum as primary antibody of the test. By using immunoperoxidase staining, 1 from 26 suspected sections of candidiasis gave a positive stain. None of 26 suspected sections of aspergillosis showed a positive result. Negative reaction was obtained from 1 section from suspected case of histoplasmosis.

2.12 The Specific Diagnostic Method of Nocardia and Related Genus

Nocardia are difficult to identify to species by their morphological, physicological and chemical characteristics. Therefore, the application of TLC and GLC techniques have been used for detection of diaminopimelic acid, mycolic acid and sugar contents in whole cells.

2.13 Production and Characterization of Hepatitis B Surface Antigen (Pre S_2 and S) Expressed from Recombinant Vaccinia Virus

From the recombinant vaccinia virus obtained from Dr.Kuniaki Nerome, NIH, Japan, the expression of Pre S_2 and S gene of Hepatitis B virus was studied by characterization of produced HBsAg.

Recombinant vaccinia virus was cultured into RK13 all line and then the expressed HBsAg was harvested from culture supernatant for characterization by reverse passive hemagglutination (RPHA), sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and electronmicroscope. The result showed that recombinant vaccinia virus could produce HBsAg at the highest antigen titer equal to 1:128 when multiplicity of infection (m.o.i.) of 0.2 was used and the antigen was harvested during 2-3 days of culture. By using SDS-PAGE, the antigen also appeared the protein bands (GP42, P39, GP36, GP33, GP29 and P25) expressed from Pre S_2 and S genes. These proteins can react specifically with monoclonal antibodies against HBsAg by using immunoblot. Futhermore, this antigen showed the spherical form of incomplete HBV particles with 15-25 nanometers in size when it was demonstrated by electronmicroscope. Thus, this recombinant vaccinia virus can express HBV gene to produce HBsAg and this expressed antigen can be further used in immunological purpose.

3. VACCINE DEVELOPMENT AND BIOLOGICAL PRODUCTION

3.1 Development of Acellular Pertussis Vaccine

Three batches of acellular pertussis vaccine were produced and tested for pH, aluminium content, thimerosal content, formaldehyde content, sterility, abnormal toxicity, mouse body weight-decreasing toxicity, mouse leukocyte-increasing toxicity, mouse histamine-sensitizing toxicity, potency and identity. Two batches of about 5,400 doses passed all tests but the other batch has the potency lower than the requirement (<8 IU/ml).

3.2 The Quality Control Tests of Pertussis Component Vaccine (Acellular Pertussis Vaccine)

Altogether five tests on the pertussis component vaccine produced in this project were conducted, they are:-

- Test for mouse body weight-decreasing toxicity
- Test for mouse leukocyte-increasing toxicity
- Test for mouse histamine-sensitizing toxicity
- Limulus amoebocyte lysate test for detection of endotoxins
- Standard preparation for toxicity test

The lyophilized B. pertussis strain 18-323 were prepared for intracerebrally challenge.

The results show that all three batches of the vaccine passed every tests. About 250 vials of lyophilized form of 100 OU. of killed B. pertussis per vial had been prepared to be used as standard preparation for toxicity tests. About 200 vials of lyophilized B. pertussis strain 18-323 for challenging in the potency test had been prepared.

The field trial of the pertussis component vaccine will be performed.

3.3 Development of Purified Chick Embryo Cell Rabies Vaccine (PCEC Rabies Vaccine)

Three batches of about 4,400 doses of freeze-dried PCEC rabies vaccine were produced and tested for sterility, toxicity, potency, stability, moisture content, protein content, pH and pyrogen. Alll three batches passed all tests and the vaccine will be used in the field trial.

3.4 Measles Vaccine Production and Control Test Training

The experience gained from this training:

1. Measles Vaccine Production
   - 1.1 Preparation of Chicken Embryo Fibroblast cell culture
   - 1.2 Inoculation of Measles Seed Viral
   - 1.3 Harvesting and Purification of Viral Suspension
   - 1.4 Preparation of Final Bulk
   - 1.5 Filling and Lyophilization
2. Control test
   - 2.1 Sterility test
   - 2.2 Test for freedom from extraneous viruses
   - 2.3 Test for virus content
   - 2.4 Identity test
   - 2.5 Test for freedom from abnormal toxicity
   - 2.6 Test for residual moisture content
   - 2.7 Test for residual animal serum protein

3. Preparation of measles antigen and antibody

The quality control on lived viral vaccine, and the preparation of measles antigen and antibody will be performed.

3.5 Production of Reference Materials for Biological Products

The following reference preparations, and standards for the control of biological products have been prepared and being calibrated:

1. Reference seed JE virus (Beijing), challenge strain 99x0.3 ml
2. Reference seed B. pertussis (18323), challenge strain 100x0.1 ml
3. Standard pertussis vaccine 250x10 ml
4. Standard diphtheria toxoid (100 Lf/ml) 255x1 ml
5. Standard tetanus toxoid (50 Lf/ml) 250x1 ml
6. Reference preparation for rabies vaccine 130x10 ml
7. Standard anti-poliovirus serum (type 1), rabbit 80 ml
8. Standard anti-poliovirus serum (type 2), rabbit 60 ml
9. Standard anti-poliovirus serum (type 3), rabbit 90 ml

4. EPIDEMIOLOGICAL STUDIES OF INFECTIOUS DISEASES

4.1 Serotyping of Nonserotypable Human Rotavirus Strains by PCR Typing

A total group of a human rotavirus (HRV) collected in Bangkok and Songkhla were examined for their serological properties on VP4, VP6 and VP7. The VP7 serotype specificity of 49 (79.4%) out of 65 HRV strains collected in Songkhla could be

determined : 42 were serotype 1, 3 were serotype 2, 4 were serotype 4 and 16 nonserotypable. Twenty one (61.8%) out of 34 of HRV strains collected in Bangkok were serotype as followed : 7 were serotype 1, 9 were serotype 2, 2 were serotype 3, 1 were serotype 4 and 13 nonserotypable. In addition, 29 stool specimens nonserotypable by ELISA could be type by PCR method. By using the PCR typing method, we used type-specific primer derived from distinct region of the gene. A characteristic segment size of each human serotype were produced by 2 times of amplification and readily identifiable in agarose gel. Twenty (68.9%) out of 29 could be serotype : 14 were serotype 1, 6 were serotype 4 and 9 nonserotypable. These results suggested that some of these nonserotypable strains might be grouped as new serotype (s).

4.2 Molecular Epidemiology of Influenza Viruses

The purified RNA of the influenza viruses was synthesized to first-stranded cDNA using reverse transcriptase and specific primer. The sequencing technique was done as Sanger's method of dideoxy-mediated chain termination. By using the four different dd NTPs, the products of the reaction are series of oligonucleotide chains that terminate at positions occupied by every A,C,G or T in the template strand. These populations of oligonucleotides are then resolved by polyacrylamide gel electrophoresis that can discriminate between individual DNAs that differ in length. When the populations are loaded into adjacent lanes of a sequencing gel, the order of nucleotides along the DNA can be read directly from an autoradiographic image of the gel.

This is the first demonstration which showed how to perform nucleotide sequencing technique. Only one specimen was used to demonstrate and there was no comparison with standard strains. In addition, we could observe some oligonucleotides which were not discriminated between single-stranded DNA differing in length. So the result could not be concluded.

4.3 Environmental Surveillance of Legionella spp in Thailand

The distribution and seasonal variation of Legionella spp. in the environment have been investigated. Legionella spp. were found in 19.6% (45/230) and 5.6% (11/197) of cooling tower water and environmental water samples. Species of Legionella will be identified by using DNA-DNA hybridization, isoprenoid quinone determination and quanine-cytocine content in a cell. The positive and negative specimens will be further determined the existence of organism by polymerase chain reaction technique.

4.4 Surveillance of Enterotoxigenic and Enteroinvasive Escherichia coli in Thailand by DNA-DNA Hybridization

This project is aimed to find the incidence of Enterotoxigenic (ETEC) and Enteroinvasive (EIEC) E. coli by using DNA-DNA hybridization. The results of which will provide the evidences of virulence factors and common serogroups of ETEC and EIEC found in Thailand. The E. coli strains isolated from diarrheal patients without the prominence of some other enteric pathogenic bacteria were submitted. Anaerogenic E. coli formerly

sent for the confirmation of Shigella were also examined. Isotopic-labeled DNA probes specific to EIEC and LT, ST1a, ST1b enterotoxins of ETEC, respectively, were hybridized with E. coli isolates. The probe positive strains were agglutinated with O and M antisera of E. coli, 17 types of O-antisera produced by DMS, another 27 types of O-antisera provided by SEAMIC/IMFJ, 22 H-antisera kindly provided by JICA. Of total 2800 strains of E. coli tested, 143 and 148 strains were positive to ETEC and EIEC probes respectively. 50% of the ETEC strains produced ST,b enterotoxin. Agglutination of all probe-positive strains with O and H E. coli antisera have not yet been accomplished. Some further examinations are required.

4.5 Biological and Biophysical Differentiation of Human Herpes Virus 6 (HHV6) Isolated from Exanthem Subitum (ES) in Thailand

This study is undertaken to characterize HHV6 from ES isolates in Thailand by their growth activities, DNA analysis and comparing with the reference strains, Z 29 and HST and strains. Growth activities of HHV6 from each isolate was studied by its replication in different T cell lines; PHA stimulated cord blood (CB) lymphocytes, MT4, MOLT4, MOLT3 and HSB2. The result showed that strains of ten isolates and reference strains had similar ability to infect these all lines. They could grow well in CB lymphocytes, MT4 but less activities in MOLT4, MOLT3 and could not grow in HSB2.

For DNA analysis, all strains looked similar in DNA patterns eventhough there were still some variations in DNA bands

among each strains when DNA were analyzed with restriction enzymes, PstI, Hind III, BamHI and ECoRI. These variations were further identified by using DNA hybridization with the selected colonal DNA probes. The results showed that the similarity of HHV6-DNA from each strain located in the terminal repeat and the unique sequence of HHV6-genome while the different parts located at the junction between these two. Morevover, the variation of HHV6-DNA could be recognized in the unique sequence of some HHV6-Thai strains. This may be from some mutation or some geographic difference.

From the study, HHV6 from ES isolates in Thailand are similar to reference strains, Z 29, HST. Therefore, HHV6 from these ES isolates can be identified into group B as the previous report of reference strains. However, it should be further studied in more strains of HHV6 to reach the possibility of identification into sub-groups.

4.6 Survey on Neonatal Congenital Hypothyroidism Using an Appropriate RI Technology in Iodine Deficiency Endemics Areas in Thailand

A pilot survey programme for Neonatal Congenital Hypothyroidism has been done in 11 provinces in Northern and Northeastern Parts of Thailand. 10,242 specimens from heels of infants, age > 48 hours, were collected by using blotting paper and mailed to NIH laboartory. Immunoradiometric assay technique was used for screening of Thyroid Stimulating Hormones in the samples

using NIH-IRMA TSH kit. Preliminary evaluation showed that the incidence of Congenital Hypothyroidism was 1:2,560 in spite of the fact that only 21% of the samples could be recalled for confirmation of the results. Thus, it was obviously seen that the National Programme for Neonatal Congenital Hypothyroidism Screening should be implemented as soon as possible in order to prevent the occuring of mental retardation babies in Thailand.

5. RESEARCH IN MEDICAL ENTOMOLOGY

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

This study aims to determine the abundance of the JE-vector mosquitoes in high and low incidence of encephalitis areas, and to evaluate the JE virus antigen in wild-caught mosquitoes.

From January to December 1991, the mosquitoes were collected by UV-light traps once a month from 3 stations in each of the 4 provinces of high and low encephalitis incidence, i.e. Uttaradit and Chiengrai represent the high-risk area, and Chumporn and Surathani represent the low-risk area. 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 medthod is required. However they are necessary to prove this hypothesis by longitudinal studies in all the regions of Thailand.

6. ESTABLISHMENT AND STRENGTHENING OF REFERENCE SYSTEM

6.1 WHO National Phage Typing Center

The use of bacteriophage typing as an epidemiological tool is advantageous for the investigation of some causative agents when the outbreaks occur. Since 1981, Phage typing of Salmonella Typhi and S. Paratyphi A were carried on routinely. In 1990, Phage typing of Staphylococcus aureus was set up. More than one thousand strains of Staph. aureus received from avarious hospitals were identified for phage type. Among these strains, nearly 60 percents are Methicillin Resistance Staph. aureus (MRSA) which could not be typed by this basic set of phage.

In the future, the production of phage group of MRSA will be undertaken. So that the nosocomial infection Staphylococci could be identified and the irradication of this causing agent could be success.

6.2 Establishment of Type and Reference Culture Collection

The clinical isolates in the country and reference strains were collected for furthur study and supplied to other laboratories upon request.

6.3 Establishment of Serum Bank

Sera were collected for epidemiological surveillance and research study.

6.4 Establishment of Rickettsial Laboratory

Rickettsial Laboratory was established in NIH, in 1990. The technique for serodiagnosis by IFA was set up. To participate in the WHO project on global surveillance of rickettsial diseases, WHO diagnostic kit for identification of anti-rickettsial antibodies has been used for detection of R. conorii and R. prowaza kit.

The antigens are being produced for diagnosis of scrub typhus (R. tsutsugamushi) using L929 tissue culture.

6.5 Establishment of WHO Regional Laboratory for Polio Diagnosis

Enteric Virus Section, Virus Research Institute, NIH, is to be designated as the Regional Reference Laboratory of the WHO Global Poliomyelitis Eradication Laboratory Network.

7. COMMON LABORATORY ACTIVITIES

7.1 Animal Experimental Center

- Routine service of the SPF Japanese Quail's Eggs
- Routine service of the experimental areas for both infectious and non-infectious aspects
- Routine supply of experimental animals, such as mice, chicken, etc.
- Training in microbiological monitoring of laboratory animals

The experience gained from this training:

Training at NIH:

1. Bacterial cause of disease in small laboartory animals including isolation and identification of some specific pathogens.
2. Study on their pathogenesis and pathological lesions by experimental infection.
3. Bordettella bronchisepticum (killed) vaccine preparation for vaccinating in guinea pig colony

Training at CIEA:

1. Management and process in microbiological monitoring used as health quality control of defined laboratory animals.
2. Serological test for detection antibody to some specific pathogens (Mouse Hepatitis Virus, Sendai Virus, Mycoplasma, Tyzzer's Organism)
3. The production of CF, HI, ELISA, IFA antigen of those pathogens (mentioned in 1) that are used in the tests.
4. The maintenance of nucleus colony of gnotobiotic animals in vinyl isolator.

Training at SLC INC:

1. The production of specific pathogen free laboratory animals in large number and its facilities.
2. Microbiological monitoring for laboratory animal quality control in commercial breeder farm.

7.2 Biohazard Laboratory

- Routine service of experimental areas for biohazard experiments and waste treatments.

7.3 Radioisotope Laboratory

- Routine service of experimental areas and supplies for both infectious and non-infectious aspects
- Management of radioisotope waste products and treatment
- Monitoring and control of radioisotope used in research activities

7.4 Scientific Equipment Centre

- Routine service of central equipment facilities and supplies
- Routine service of repairing of scientific equipment and installation of new equipment in both central and regional laboratories
- Routine service of public utilities and supplies for NIH building
- Routine service of computer applications and programme development

8. AIDS

8.1 Activities of AIDS Prevention and Control Programme

1. External Quality Assurance in HIV Blood Testing.

NIH is the organizing laboratory responsible for external quality assessment of HIV blood testing. A target of 220 hospital laboratories all over the country will participate in this programme. The programme was started in 1990. In 1991, about 100 laboratories have been participated. The panel sera are sent to the laboratories twice a year. (Supported by the World Health Organization)

2. Training Laboratory Personnel in HIV Blood Testing.

NIH and Regional Medical Science Centers held work-shops on laboratory diagnosis of HIV infection in 1991. About 200 laboratory personnel participated.

3. Characterization of HIV isolates in Thailand.

In 1991, upon the collaboration of the NIH Thailand, NIH Japan and Centers for Disease Control (C.D.C.), U.S.A., HIV-1 genetic characterization was performed on 21 representative specimens from HIV-1 infected persons by sequencing and/or hybri dization at C.D.C., U.S.A.. Nucleotide sequence of the V3 domain showed two distinct HIV-1 subtypes, A and B. Subtype A appeared to be present in every risk group examined, while subtype B appeared to be present primarily in IVDU. Biological study of HIV isolates was performed by virus isolation of the total 16 samples: 13 samples from asymptomatic HIV carriers and 3 from AIDS patients. Four samples of the carriers showed positive

signal by reversetranscriptase (RT) assay and ELISA antigen detection. No syncytium formation was observed during cocultivation with normal peripheral blood mononuclear cells. Polymerase Chain Reaction (PCR) assay for the detection of HIV proviral DNA was performed in IVDU's blood samples. Three regions of HIV-DNA, GAG, ENV and LTR, were amplified and were detected by hybridization using nonisotopic DNA probe. From 26 blood samples of IVDU, GAG gene was found in 8 samples (31%), ENV gene in 20 samples (77%) and LTR gene in 9 samples (35%). ENV gene seems to be the most common for the detection of HIV proviral DNA in this IVDU group. It was not able to isolated the virus from those samples by co-cultivation with normal PBMCs from cord blood.

4. Laboratory Services for HIV Diagnosis and Sentinel Serosurvey of HIV Infection in Thailand.

NIH and 6 Regional Medical Science Centers have served the hospital laboratory for confirmation of the positive HIV blood screening. Since 1990, the sentinel HIV serosurvey among the risk groups in 73 provinces has been carried out twice a year by provincial hospitals and regional laboratory centers with the assistance of Department of Medical Sciences, Department of Communicable Disease Control and Epidemiology Division, Office of the Permanent Secretary of the Ministry of Public Health.

8.2 Biological and Immunological Study of HIV Isolated in Thailand

The purpose of this study is to isolate and characterize HIV strain circulating in Thailand. The process is as following:

- Separate peripheral blood mononuclear cell (PBMCs)
- Coculture a half of whole PBMC with PHA strinulated normal PBMC
- Separate CD rich fraction from another half of whole PBMCs and coculture with PHA stimulated normal PBMC
- Keep the culture for 4-6 weeks
    - Change the medium once a week
    - Detect virus replication by Reverstranscriptase assay (RT) and IFA at 1 week interval
    - Maintain the culture by adding fresh normal PBMCs

The total of 16 samples, 13 samples fronples from asymptomatic HIV carrirs (AC) and 3 from AIDS patients, was attempted for HIV isolation. By using cocultivation with normal PBMCs, 1 sample of AC showed positive signal by RT assay and IFA. It was kept at -85C as stock for furthur characterization.

Approximately 40 of HIV srains are expected to be isolated from asymptomatic carrier and AIDS patients by using cocultivation with normal PBMCs and CD rich fraction in order to characterize in the point of similarity and dissimilarity of HIV strains circulating in Thailand. Biological study involved cell tropism, cell killing activity, syncytium formation activity, amplification of important HIV gene by PCR. The outcome of those basic information related to HIV isolated in Thailand are likely to be potentially useful for selection of AIDS vaccine and specific markers that related with pathogenicity, transmissibility and epidemiology of HIV.

8.3 Future Plan

1. Some activities must be continued as a routine activity and being expanded to the large scale operation such as:

   - Quality Assurance in HIV Blood Testing
   - Training
   - Laboratory services and sentinel serosurvey of HIV infection.

2. Survey of opportunistic infection of AIDS patients in Thailand. The activities will include the strengthening of laboratory capabilities, production of the manuals, training and monitoring the opportunistic diseases of AIDS.

3. Characterization of HIV isolates in Thailand.

4. Production of diagnostic reagents for HIV infection such as antigen detection and rapid test for antibody detection.

9. TRAINING PROGRAM

Many training programs were provided to both local and overseas participants of which the lists are shown on the following pages.

Training to Thai Participants
From December 1990 - January 1992

No.	Subject/Field	Duration (day/month)	No. of Participants
1	DHF Vector Surveillance and Control	3 days	49
2	Training Course on DHF and JE Serodiagnosis	8 days	23
3	Viral Serology; HAV, HBV Infection; Respiratory Viruses Herpes Virus and Rubella	2 1/2 days	33
4	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	2 days	2
5	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1 days	30
6	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1/2 days (10 times)	213 (21,3,20,20 36,15,35, 22,15,26)
7	Development of HB Vaccine from Yeast	1/2 days	5
8	Training Course on Phage Typing Technique of Staphylococus Aureus	5 days	10
9	Technical Training Course on Gastrointestinal Infections	5 days	20
10	Technology Transfer in the Confirmation of Shigella to Regional Medical Sciences Centers	5 days	6
11	Analysis of Thyroid Function Hormones by Immunoassay Techniques	5 days	12
12	Advance on Laboratory Diagnosis for HIV Infection	3 days	20
13	Laboratory Animal Sciences for Assistant Veterinarian	9 months	180

Training to Thai Participants
From December 1990 - January 1992

No.	Subject/Field	Duration (day/month)	No. of Participants
14	An Introduction to Laboratory Animal Sciences and Its Facilities	1 day	40 (Veterinary Medicine Students)
15	Microbiological Monitoring System for Health Quality Control in Laboratory Animal Production	1 day	200 (Special lecture on Work for Biological researchers)
16	SPF Laboratory Animal (Specific Pathogen Free)	1 day	200 (Special lecture on work for biological researchers)
17	Using Analytical and Research Instruments	5 days	28
18	Training Computer Course on DMBS Programme	3 days	16

Training to Overseas Participants
From December 1990 - January 1992

No.	Subject/Field	Duration (day/month)	No. of Participants	Country
1	Quality Control of Measles Vaccine	1 month	2	Vietnam
2	Quality Control of Biological Products	5 days	1	China
3	Epidemiology of Dengue Fever	2 months	1	China
4	Production Techniques of Antigen and Laboratories Diagnosis of Dengue Fever	2 months	2	Laos
5	Enterovirus	1 day	2	Sri Lanka
6	Enterovirus	1 day	1	China
7	Enterovirus	1 day	2	Myanma
8	Enterovirus	1 day	1	Sri Lanka
9	Japanese Encephalitis	3 days	3	India
10	Epidemiology of Dengue Fever and Its Vector Control	2 months	1	China
11	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1/2 day	5	India
12	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1 day	10	Japan
13	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1/2 day	2	Indonesia
14	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1/2 day	3	Vietnam
15	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1 day	1	Republic of China

Training to Overseas Participants
From December 1990 - January 1992

No.	Subject/Field	Duration (day/month)	No. of Participants	Country
16	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1 day	1	Sri Lanka
17	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1 day	2	Myanma
18	Viral Hepatitis, Respiratory Viruses, Herpes Virus and Rubella	1/2 day	2	Vietnam
19	Respiratory Viruses, Herpes Virus and Rubella	2 days	2	Vietnam
20	Viral Hepatitis	1/2 day	12	Participants of 2nd Asia Pacific Congress of Medical Virology
21	Viral Hepatitis	2 days	2	Laos
22	Production of Radioimmuno Assay Reagents	5 days	2	Myanmar Sri Lanka
23	Quality Assurance in HIV Blood Testing	5 days	1	Mongolia
24	Laboratory Diagnosis for HIV Infection	1 days	1	Sri Lanka

Work Plan for 1992/1993

Virology

1. Development of new diagnosis technique on viral disease including production and quality control of HIV test kit.
2. Research and development on production of diagnostic test kits by using RIA and biotechnology technique.
3. Immunological and biological characterization of HIV isolation in Thailand.
4. Study on medicinal plant extract for anti HIV.
5. Research and development on Hepatitis B. diagnosis from Serum.
6. Research on diagnosis of Rickettsial disease and production of test kit.
7. Study on impacts of the national JE vaccination in Thailand.
8. Genetic different of poliovirus between vaccine strain and wild strain.
9. Serological and genetical characterization of rotavirus in different localities in Thailand.
10. Epidemiological study of cervical cancer from human papilloma virus in Thailand.

Bacteriology, Mycology and Parasitology

1. Research and development on production of diagnostic test kits.
2. Develop new diagnosis technique of bacterial diseases.
3. Epidemiological study on phage type, plasmid profile of infectious diseases.

Biological Products

1. Development of Diphtheria Toxoid by fermentation techniques.
2. Strengthening and development on quality control of biological products.
3. Study on cold chain system of vaccine in EPI program.
4. Production of freeze-dried National Standard Biological Products.

Medical Entomology

1. Study on susceptibility and rate of resistant against insecticide.
2. Research on JE and dengue vector surveillance
3. Study on efficiency of insecticide and its applicator
4. Study on bioefficacy of household insecticide
5. Control of larvae of mosquito by using particular fish.
6. Method development for bacterial control product of mosquito larvae.

Others

1. Research on prevention and control of iodine deficiency disorder.

Manpower of NIH 1991

Division	Scientific Staff				General Administrative Staff				Sub Total	Workers	Total
	Ph.D.	Master	Bachelor	Others	Ph.D.	Master	Bachelor	Others			
Office of the Secretary	-	-	-	-	-	-	5	15	20	3	23
Biological Products Division	-	1	10	3	-	-	1	5	20	5	25
Clinical Pathology Division	1	10	15	16	-	-	-	6	48	21	69
Health Science Research Institute	-	13	17	5	-	-	1	16	52	29	81
Medical Entomology Division	-	10	7	7	-	-	1	7	32	29	61
Medicinal Plant Research and Development Division	3	13	20	8	-	1	1	11	57	32	89
Virus Research Institute	-	6	23	13	-	-	1	9	52	22	74
	4	53	92	52	-	1	10	69	281	141	422
	201				80						

Ph.D.	4	= 1.4%
Master Degree	54	=19.2%
Bachelor Degree	102	=36.3%
Others	121	=43.1%
Total	281	= 100%

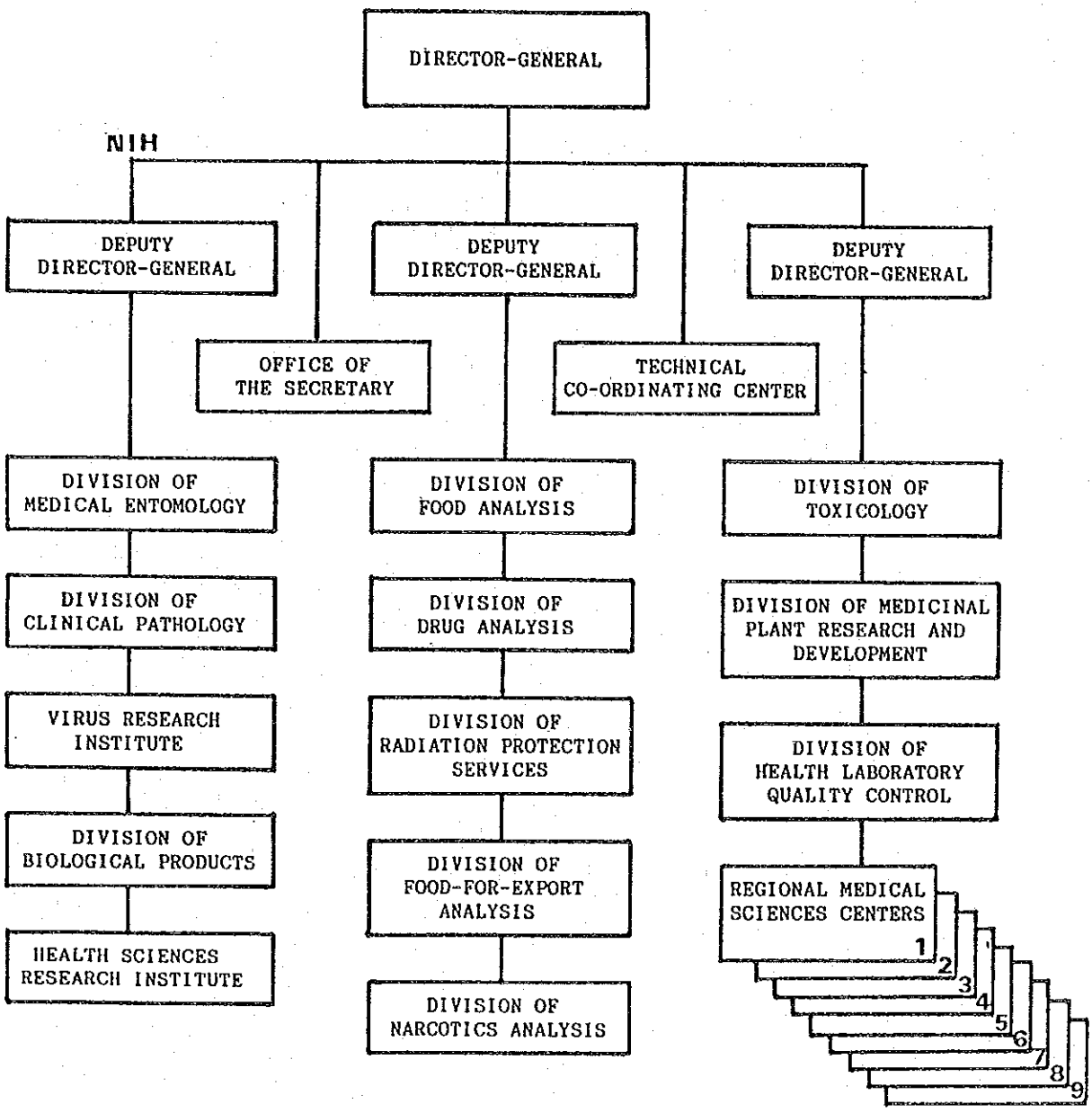
Source of NIH Fund 1991

91 90

Source of Fund		Budget (Baht)
Government Budget		51,719,000.00
Others		3,212,617.90
Foreign Aid (WHO, IDRC)		1,697,477.00
	Sub Total	56,629,094.90
X JICA		4,170,000.00
	Total	60,799,094.90

ORGANIZATION CHART

DEPARTMENT OF MEDICAL SCIENCES



Steering Committee 1991

1.	Khunying Preeya Kashemsant Director-General, DMS	Honorable Consultant
2.	Dr. Nadhirat Sangkawibha	Honorable Consultant
3.	Dr. Sompop Ahandrik Deputy Director-General, DMS (Director, NIH)	Chairman
4.	Dr. Boonluan Phanthumachinda Inspector-General, MOPH	Member
5.	Dr. ML. Ratanasuda Phan-urai Principal Medical Officer	Member
6.	Dr. Chuinrudee Jayavasu Principal Medical Officer	Member
7.	Dr. Mayura Kusum Director, Division of Clinical Pathology	Member
8.	Dr. Paijit Warachit Director, Virus Research Institute	Member
9.	Mr. Prakong Phan-urai Director, Division of Medical Entomology	Member
10.	Mrs. Kanchana Leelasiri Director, Division of Biological Products	Member
11.	Dr. Jakkriss Bhumixawasdi Director, Health Sciences Research Institute	Member
12.	Mr. Kamol Sawasdimongkoi Director, Division of Medicinal Plant Research and Development	Member

13. Dr. Komi Kanai — Member
    Japanese Project Leader
14. Mr. Kohei Nakajima — Member
    Japanese Coordinator
15. Japanese Experts — Member
16. Dr. Chongdee Wongpinairat — Member and Secretary
    Director, Technical Coordinating Center
17. Mrs. Siripan Wongwanich — Assistant Secretary
    Division of Clinical Pathology

Coordinating Committee 1985 up to Present

1.	Permanent Secretary Ministry of Public Health	Chairman
2.	Dr. Nadhirat Sangkawibha	Honorable Consultant
3.	Director General, DMS	Member
4.	Deputy Director-Generals, DMS	Member
5.	Principal Medical Officers	Member
6.	A representative of the University Affairs Office	Member
7.	A representative of the Department of Technical and Economic Cooperation	Member
8.	Dr. Komi Kanai Japanese Project Leader	Member
9.	Experts (dispatched by JICA)	Member
10.	Resident Representative of the Bangkok Office, JICA	Member
11.	Mr. Kohei Nakajima Japanese Coordinator	Member
12.	Dr. Sompop Ahandrik Deputy Director-General, DMS (Director, NIH)	Member and Secretary
13.	Dr. Chongdee Wongpinairat Director, Technical Coordinating Center	Member and Assistant Secretary
14.	Miss Wiyada Charoensiriwatana Health Sciences Research Institute	Assistant Secretary

③　カウンターパート研究発表アブストラクト

ABSTRACTS

RESEARCH ACTIVITIES
OF THE
RESEARCH PROMOTION PROJECT
NATIONAL INSTITUTE OF HEALTH
DEPARTMENT OF MEDICAL SCIENCES
NONTHABURI, THAILAND

PRESENTATION

TO

THE JAPANESE FINAL EVALUATION TEAM

JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)

28 February - 2 March, 1992

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Serotyping of Nonserotypable Human Rotavirus Strains by PCR Typing

Yaowapa Pongsuwanna

Virus Research Institute, Department of Medical Sciences

A total group of a human rotavirus (HRV) collected in Bangkok and Songkhla were examined for their serological properties on VP4, VP6 and VP7. The VP7 serotype specificity of 49 (79.4%) out of 65 HRV strains collected in Songkhla could be determined : 42 were serotype 1, 3 were serotype 2, 4 were serotype 4 and 16 nonserotypable. Twenty one (61.8%) out of 34 of HRV strains collected in Bangkok were serotype as followed : 7 were serotype 1, 9 were serotype 2, 2 were serotype 3, 1 were serotype 4 and 13 nonserotypable. In addition 29 stool specimens nonserotypable by ELISA could be type by PCR method. By using a PCR typing method, we used type-specific primer derived from distinct region of the gene. A characteristic segment size of each human serotype were produced by 2 times of amplification and readily identifiable in agarose gel. Twenty (68.9%) out of 29 could be serotype : 14 were serotype 1, 6 were serotype 4 and 9 nonserotypable. These results suggested that some of these nonserotypable strains might be grouped as new serotype (s).

The Complete Nucleotide Sequence of a Variant of Coxsackievirus A24

Kasana Supanaranond

Virus Research Institute, Department of Medical Sciences

A variant of Coxsackievirus A24 (CA24v) is one of the agents causing of acute hemorrhagic conjunctivitis (AHC). Since CA24v emerged as a new human pathogen from a single focus, like enterovirus 70 (EV70), the virus provides us a unique example to find a clue to indentify the origin of the virus as well as to know the evolutionary relationship among other picornaviruses, we have determined the complete nucleotide sequence of the standard strain of CA24v, the EH24/70 strain.

The genome of CA24v is 7,461 nucleotides long with poly (A) tail at 3' end. Following a 750 nucleotides 5' non-coding region, there is long open reading frame of 7,392 nucleotides, which serve to encode a viral polyprotein consisting of 2,214 amino acids. Comparison of the deduced amino acid sequence the polyprotein with that of known enteroviruses allowed us to predict the possible cleavage sites.

The overall structure and the organization of the RNA genome is typical for an enterovirus. Based on the similarity of the nucleotide sequence of the 5' and 3' non-coding regions together with the amino acid sequence of the encoded proteins, EH24/70 appeared to be closely related to poliovirus and coxsackievirus A21.

Molecular Epidemiology of Influenza Viruses

S. Pattamadilok

Virus Research Institute, Department of Medical Sciences

During 1983-1990, laboratory surveillance of influenza in Bangkok was carried out on the basis of virus isolation. It was found that there were antigenic transition of the influenza isolated in a period of that time. Epidemiological studies at the molecular level would be very helpful to understand the genetic behavior and to predict the future epidemic of influenza viruses. According to this concept, nucleotide sequencing technique had been demonstrated by Dr. Nerome from National Institute of Health, Japan. Briefly, the purified RNA of the influenza viruses was synthesized to first-stranded cDNA using reverse transcriptase and specific primer. The sequencing technique was done as Sanger's method of dideoxy-mediated chain termination. By using the four different dd NTPs, the products of the reaction are series of oligonucleotide chains that terminate at positions occupied by every A,C,G or T in the template strand. These populations of oligonucleotides are then resolved by polyacrylamide gel electrophoresis that can discriminate between individual DNAs that differ in length. When the populations are loaded into adjacent lanes of a sequencing gel, the order of nucleotides along the DNA can be read directly from an autoradiographic image of the gel.

This is the first demonstration which showed how to perform nucleotide sequencing technique. Only one specimen was used to demonstrate and there was no comparison with standard strains. In addition, we could observe some oligonucleotides which were not discriminated between single-stranded DNA differing in length. So the result could not be concluded.

Molecular Diagnosis of Dengue Viruses Using RT-PCR Technique

S. Rojanasuphot and S. Vongcheree

Virus Research Institute, Department of Medical Sciences

Diagnosis of dengue viruses using RT-PCR was developed in Arbovirus unit NIH, DMS. The technique was tested with Rayong samples, in comparison with virus isolation method. From twenty eight positive virus isolated samples, there were twenty two samples which were completely coincided by RT-PCR. Furthermore, the techique was rapid enough to detect and identify the viruses within two-day test, and various forms of samples could be analysed with or without sample extraction. Some details of the technique might be further studied before using it as diagnostic method for dengue virus infection in human.

Preparation of Dengue and JE Antigens for Antibody Capture ELISA

S. Rojanasuphot and S. Pothipunya

Virus Research Institute, Department of Medical Sciences

The optimal conditions for dengue and JE virus antigens from tissue culture were innovated. Twelve strains of dengue viruses serotypes 1 to 4 and two strains of JE viruses from different sources were cultured in C6/36 cells. Firstly, a more versatile mosquitoes cell line was replaced the former one. Secondly, various virus seeds were studied to find out the proper sources. Finally, the antigens from tissue culture were daily checked for HA unit and ELISA unit for ten days interval in order to find out the maximal dates for each viruses. The optimal HA titers of these antigens were at 6-10 days, 2-4 days and 4-10 days post infection for dengue serotypes 1 and 2, serotypes 3 and 4 and JE viruses, respectively. The optimal ELISA titers were at 10 days, 4-10 days, 4 days, 2-4 days and 4 days post infection for dengue serotypes 1,2,3,4 and JE viruses, respectively.

Enzyme Immuno Assay for the Detection of Rabies Neutralizing Antibody

P. Samuthananon

Virus Research Institute, Department of Medical Sciences

By the coperation with Japanese expert, we have developed an EIA technique for detection of rabies neutralizing antibody has been developed. Forty immunized sera were studied by using this technique. The results compared to those of the standard mouse neutralization test, show no statistical significant difference.

It has proved that, EIA is a specific, convenient and useful method for the quantitative assay of rabies virus neutralizing antibody.

An IgM ELISA for the Serodiagnosis of Poliovirus Infection

Kasama Supanaranond[1] and Osamu Nishio[2]

[1] Virus Research Institute, Department of Medical Sciences

[2] Lab. of Virology, Aichi Prefectural Institute of Public Health, Nagoya, Japan

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In 1988, the World Health Assembly committed the World Health Organization to the global eradication of poliomyelitis by the year 2000. Current method used for serodiagnosis of poliovrus infections and for seroepidemiological studies are still based on tissue culture techniques. They are time consuming. An IgM ELISA, rapid and simple test, for detecting poliomyelitis IgM antibody for the diagnosis of poliovirus infection was developed. The method has no cross reaction with other enteroviruses. When we compared the positive rate between Microneutralization test, the serological test of choice, and the IgM ELISA test, the latter was found to be more sensitive. From 49 suspected poliomyelitis cases, the positive rate of microneutralization test was 40.8% and IgM ELISA test was 67.3%.

Biological and Immunological Study of HIV Isolated in Thailand

Wattana. Auwanit

Health Science Research Institute, Department of Medical Sciences

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Microbiological Monitoring of Laboratory Animals

Raywadee Butraporn

Health Science Research Institute, Department of Medical Sciences

Development of Pertussis Component Vaccine

Teeranart Jivapaisarnpong, Prayute Buddhirakkul

Division of Biological Products, Department of Medical Sciences

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Production of Chick Embryo Cell Culture Rabies Vaccine

Prakorb Ruenrairatanaroj

Division of Biological Products, Department of Medical Sciences

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In pilot study on production of PCEC rabies vaccine, six lots of the vaccine were produced as the following method. The primary chick embryo cell was cultured from the 7-day old chick embryonated egg and the rabies virus, HEP flurry strain was, inoculated into this cell culture. On the 6th, 11th and 16th days after inoculation, the culture fluid was harvested and inactivated by adding 1:2500 betapropiolactone. The inactivated virus suspension was concentrated and purified by ultrafiltration and ultracentrifugation, respectively. Finally, the vaccine was lyophilized and the quality control tests of the lyophilized vaccine were performed according to WHO requirements. All six lots (4400 doses) of the vaccine produced passed all quality control tests.

Application of Immunofluorescent Microscopy to Rapid Diagnosis of Melioidosis

Pimjai Naigowit[1], Vimon Petkanjanapong[1], Takeshi Kurata[2], Eiko Kondo[2] and Komi Kanai[2]

[1] Division of Clinical Pathology, Department of Medical Sciences

[2] National Institute of Health, Japan

Melioidosis caused by Pseudomonas pseudomallei is a serious infectious disease whose endemic areas are present in all the provinces of Thailand. The motality rate was as high as 83.7% in the septicemic cases. The morbidity rate is increasing every year. Because of the necessity of rapid diagnosis for the treatment of this disease, an attempt was made to develop indirect immunofluorescent microscopy with LPS or a protein fraction separated from P. pseudomallei as antigens. The antisera to these antigens were prepared using rabbits and guinea pigs. The specificity and sensitivity of our test system in the detection of the antigens was examined using 18 melioidosis specimens and 44 non-melioidosis specimens.

Fifteen (83.3%) out of 18 melioidosis samples diagnosed from clinical symtoms and culture-positive gave fluorescence-positive results. Six out of 44 non-melioidosis samples showed positive or unclear fluorescence.

Cross reaction in this immunofluorescent microscopy was examined between P. pseudomallei (ATCC type strain and clinical

isolates) and other species (P. cepacia and E. coli). No cross reaction was observed when the anti-LPS guinea pig sera was employed, but the anti-LPS rabbit sera gave weak cross reaction.

From these results, we are encouraged to think that the immunofluorescent microscopy is useful as the rapid diagnostic method. The introduction of monoclonal antibody is now under consideration to improve the specificity of this method.

Immunofluorescent Microscopy for the Detection of IgM and IgG Antibodies to Pseudomonas pseudomallei

Pimjai Naigowit[1], Vimon Petkanjanapong[1], Takeshi Kurata[2], Eiko Kondo[2] and Komi Kanai[2]

[1] Division of Clinical Pathology, Department of Medical Sciences

[2] National Institute of Health, Japan

Sera from 61 melioidosis patients, 37 other septicemic diseases and 80 healthy individuals were examined for the presence of IgM and IgG antibodies to Pseudomonas pseudomallei by indirect immunofluorescent antibody (IFA). From this study IFA-IgM titer > 1:8 and IgG > 1:64 were diagnostic value for melioidosis. The sensitivity, specificity and accuracy of IFA-IgM and IFA-IgG were 90.2%, 100.0%, 95.7% and 95.1%, 100.0%, 97.9% respectively.

The IFA test is rapid and reliable for the detection of P. pseudomallei antibody which may be value in differentiating acute and chronic melioidosis patients from other diseases.

Extraction of Bacterial Lipopolysaccharides from *Pseudomonas pseudomallei* for Immunodiagnosis of Melioidosis by Enzyme Linked Immunosorbent Assay

Pimjai Naigowit [1], Vimon Petkanjanapong [1], Eiko Kondo [2] and Komi Kanai [2]

[1] Division of Clinical Pathology, Department of Medical Sciences

[2] National Institute of Health, Japan

Lipopolysaccharide antigen is a major component of the outer membrane of *Pseudomonas pseudomallei*, which is extracted from heated cells by liquid phenol. With the lipopolysaccharide antigen prepared, a survey was made to measure the antibody level of 47 melioidosis sera, 55 non-melioidosis sera and 50 sera of healthy donors by enzyme linked immunosorbent assay (ELISA). The sensitivity, specificity and accuracy at ELISA cut off value > OD 320 were 95.7%, 94.2% and 94.7%, respectively.

The ELISA test is technically more rapid and reliable for the detection of antibody response to *P. pseudomallei*. It may be also of value in differentiating melioidosis from other infectious diseases.

Purified Toxins of Clostridium difficile and Related Organisms

Siripan Wongwanich and Eiko Kondo

Division of Clinical Pathology, Department of Medical Sciences

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Toxins from the causative agents of diarrheal diseases, Clostridium difficile, C. sporogenes and Klebsiella oxytoca have been purified by using ammonium sulfate precipitation and DEAE-sepharose column chromatography. Four major peaks were obtained from both C. difficile A 4897 standard strain and CD11 isolated strain. K. oxytoca also gave four major peaks with different pattern form C. difficile. For C. sporogenes, only two major peaks have been shown. Some major peaks from C. difficile have strong cytotoxic activities, but the other peaks have shown haemagglutinated properties. Anti-toxin production from those purified toxins would be done for diagnostic test in diarrheal specimens.