e-ASIA Joint Research Program
Final Report

1. Project title : Development of an innovative diagnostic system against liver fluke infection in Southeast Asian countries

2. Joint Research period : Apr 1, 2015 ～ Mar 31, 2019

3. Research Team :

■ **Japan team** (up to 6 people including the Principal Investigator)
Funding period: Jun 1, 2015 - Mar 31, 2019
Total Funded Amount (in Local Currency): 26,118,280 Yen

	Name	Position	Affiliation	Role in the project
PI	Hisashi Narimatsu	Invited Senior Researcher	National Institute of Advanced Industrial Science and Technology	Director
Collaborator	Masanao Miwa	President Prof.	Nagahama Institute of Bio-Science and Technology	Design and support of measurements using clinical samples
Collaborator	Norie Araki	Assoc. Prof.	Kumamoto Univ.	
Collaborator	Atsushi Kuno	CTO	GL-i Co.	
Collaborator	Atsushi Matsuda	Assis. Prof	Keio Univ. School of Medicine	
Collaborator	Kiyohiko Angata	Senior Researcher	GL-i Co.	Support for international research
Total number of participants including students: 8				

■ **Thai team** (up to 6 people including the Principal Investigator)
Funding period: Month, Day, Year - Month, Day, Year
Total Funded Amount (in Local Currency):

	Name	Position	Affiliation	Role in the project
PI	Sopit Wongkham	Professor	Khon Kaen University	Thai leader
Collaborator	Chaisiri Wongkham	Assoc. Prof.	Khon Kaen University	Collection and measurements using clinical samples
Collaborator	Puangrat Yongvanit	Assoc. Prof.	Khon Kaen University	
Collaborator	Ubon Cha-on	Lecturer	Khon Kaen University	
Collaborator	Kulthida Vaeteewoottacharn	Lecturer	Khon Kaen University	
Collaborator	Atit Silsirivanit	Lecturer	Khon Kaen Univer	

			sity	
Total number of participants including students: 8				

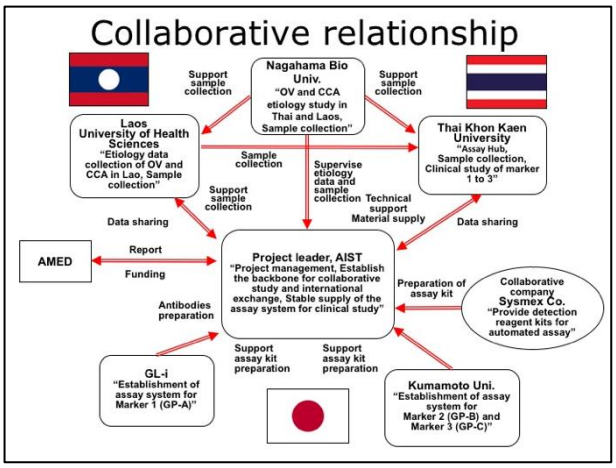
■ **Lao team**（up to 6 people including the Principal Investigator）
Funding period: Month, Day, Year – Month, Day, Year
Total Funded Amount (in Local Currency):

	Name	Position	Affiliation	Role in the project
PI	Bounthome Samountry	Vice Dean, Professor	University of Health Sciences	Lao leader
Collaborator	Khamla Phonsayalinkham	Lecturer	University of Health Sciences	Collection of clinical samples
Collaborator	Sonxay Kidoykhammouan	Lecturer	University of Health Sciences	
Total number of participants including students: 3				

4. Summary of the joint research

Cholangiocarcinoma (CCA) is known as intractable cancer with poor prognosis due to complex tumorigenesis and progression variation. In Japan, CCA accounts for under 10% of liver cancer, but almost 90% in the Mekong region including Thailand and Laos. In Thailand, CCA is the most serious cancer and about 30,000 patients die from CCA per year. CCA slowly progresses without obvious symptoms at the early stage, making late start for treatments. However, the cure rate can be increased if an early detection and clinical intervention is possible. This international research aims to develop and provide an innovative diagnostic system for quantitative and low invasive evaluation of the progress or risk of CCA associated with liver fluke infection by using leading glycotechnologies.

This project was started in June 2015. As the main objective of the first year, we focused on construction of the basis for international communications and collaborative research as well as mutual agreement on the task and establishment of the project plan. At the kick-off meeting, we agreed on the mission and goal of the project. Moreover, details of the Japanese and Thai marker candidates, GP-A, GP-B and GP-C, respectively, were presented, and the initial comparative study was planned and conducted. The collaborative research contract and other paper work were finalized early in the second year.



The manual assay technique of the marker GP-A established by AIST team was transferred to Thai researcher. And the efficiency of two markers, GP-B and GP-C, established by KKU was determined by blind ELISA assay. As the results of pre-experiments and the manual assay of additional samples, the effectivity of GP-A was confirmed by measuring both Japanese and Thai CCA samples. On the other hand, because GP-A, GP-B and GP-C have different characteristics, the effectivity of combination assay is highly expected. To establish the assay hub in KKU, various processes were made and approved in early 2016, and the automated immunoassay system was installed in KKU. Newly collected 354 Thai serum samples ethically approved by KKU were measured for the content of GP-A along with four other indices. As a result, discrimination ability for CCA was significantly high in GP-A compared to an existing cancer biomarker.

In the third year, five markers (GP-A, M2BP-Gi, CA19-9, HBVAg, HCVAb) measurement using an automatic machine were performed. Comparing Japanese markers analyzed by the machine and Thai markers (GP-B and GP-C) by ELISA with Thai samples, the analysis showed that GP-A is elevated in both Thai and Japanese CCA patients, in contrast to healthy persons. This

result suggested the applicable of GP-A for diagnosis of Thai CCA.

Thai team established 2 monoclonal antibodies (mAb), anti-GP-B and anti-GP-C antibodies, which recognize the sugar epitopes on mucin. However, these 2 mAbs are IgM and may be difficult to develop further utilities in automated system. Therefore, Japanese members suggested to switch these IgM mAb to IgG. Thereafter, the obtained IgG1 Abs were characterized by AIST researchers and now, the antibodies are further confirmed by using CCA cell lines by KKU team.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

To establish clinical biomarker detecting CCA in the Mekong region, we tested Thai and Japanese candidate biomarkers for CCA. First, the efficiency of two markers, GP-B and GP-C, established by KKU, was determined in blind ELISA assay. The manual assay technique of the marker, GP-A, established in AIST was transferred to Thai researcher by visiting AIST. Accordingly, Thai team measured the three marker candidates and statistically analyzed with a total of 428 samples (ethically approved Thai samples). In the results, the Japanese marker showed favorable results in detection of CCA with similar tendency in both Japanese and Thai samples. The two KKU markers, GP-B and GP-C, showed good results in the Thai samples and have characteristics different from those of the Japanese marker, indicating an advantage of combinational use. Furthermore, since it is necessary to apply the Thai markers for automated machine, Japanese team generated IgG versions from the Thai monoclonal antibodies.

This project was started in June 2015, and we made several scientific meetings and a symposium to establish clinical biomarker useful in Thai and Laos. The preliminary meeting was held on July in Kumamoto, Japan, to discuss the situation and solution for establishment and implementation of the assay hub. In November, 2015, all collaborators (5 from Japan, 3 from Thailand, and 3 from Laos) gathered at AIST, Tsukuba in Japan, for the kick-off meeting and agreed on the mission and goal of the project.
The first annual meeting was held in Khon Kaen University (KKU), Thailand, and University of Health Sciences (UHS), Vientiane, Laos in February, 2016.
In November 2016, all the members gathered in Kobe, Japan, for the second annual meeting. Progress of the study was reported, and a lecture was given by the Lao team leader regarding the healthcare system in Lao P.D.R.
In the second annual meeting, the antibody optimization was planned to be performed as a part of the educational activity to the young researchers. Three members from KKU have visited AIST to perform Ig class switching under supervision of AIST researchers for 2 weeks (during March 11-25 under the supported from e-Asia). In addition, a candidate for the government-sponsored foreign student was endorsed from KKU as a part of the educational activity of the project.
In January, 2018, KKU hosted the third annual meeting and opened symposium “Current Glycosciences and Glycotechnology: Application for Medicine”.

5-2 Synergistic effects of the international joint research

In this project, the international teams from Japan, Thailand, and Laos share a common goal of developing a diagnostic system that contributes to cancer prevention in patients with liver fluke infection in the Mekong River basin. To achieve this goal, we conducted side-by-side clinical studies of each developed marker from AIST and KKU, which can be achieved only by international joint research. Rather than aiming to give priority to the interests of the home country, we were able to work together under the common idea “what is truly clinically useful”. In order to achieve this goal, Laos is actually a big participant. Laos is adjacent to the Mekong River, and the number of patients infected with liver fluke is at the same level as Thailand. On the other hand, it was understood by visiting Vientiane's universities and medical institutions that there is a big difference in the medical circumstances between two countries. In Laos, the National University of Health Sciences (UHS) is the only doctor training school and Prof. Samountry is the only pathology professor in the country. Therefore, all pathologists in domestic hospitals have been trained under Prof. Samountry.

He was able to focus on the epidemiological study on hepatic fluke infection in Laos, and to clarify the difference between Thailand and Laos in the care system from infection to carcinogenesis. These observations greatly changed the image of “what is truly clinically useful”, and we experienced these observations only through this e-Asia joint research program. Vientiane, where the UHS of Laos is located, has a close relationship with Khon Kaen, a city in northeastern Thailand. Long time exchange between Prof. Wongkam and Prof. Samountry, helped to carry out this project with Japanese researchers.

5-3 Broader impacts including contribution to society

Leaders from each country promoted the participation of young researchers in the project. At the annual meeting, young researchers at the host institution took care of visiting researchers from other countries. This mutual exchange enhanced research discussions on various issues from the research associated with this project to glycoscience and glycotechnology. The effect can be seen in the status of participation in international conferences. Japan's leader, Dr. Narimatsu, is the founder of Asian Communication of Glycoscience and Glycotechnology (ACGG, https://acgg.asia). In Thailand, there were few glycoscience researchers without an academic society for glycoscience. The number of participants of ACGG from Thailand, such as Bangkok and Khon Kaen, is increased after this project, especially from Khon Kaen University. In addition, the lecturer Dr. Silsirivanit is working to establish the Glycochemical Society in Thailand. Thailand plays a central role in research on CCA and glycans (mainly focusing on clinical aspects). Recently, the contribution to the glycan research field in Asia by Thai researchers is growing further.

5-4 Development and sustainability of the cooperation

This project has promoted research that contributes to a common exit,

namely the development of diagnostic system of CCA in Thailand and Laos. Since the mission was clear, there is no change in the direction of the project after the end of the implementation period. Because we have got new IgG antibodies, which will be tested for Thai CCA samples and if it works for automated machines. Furthermore, we still need to test the combination assay of candidate markers using Thai and Lao samples. We are working on the further cooperation and seeking the financial support. The ACGG framework will also help to that end. For example, glycan researchers at Shanghai Jiao Tong University in China are interested in and contacted researchers at KKU. Thus, we expect that researchers interested in the core research themes of CCA and glycans will continue to be incorporated.

6. Future Goals and Plan of Activities after the project period

As described above, this project is considered to be a highly sustainable research topic. This activity is moving toward practical application, and not only the basic aspects described above, but also activities involving companies in each country will be desired. At present, it is only supported by Japanese companies, but in the future, Thai companies should actively participate. We believe this is an important action for bringing Japanese technology to Thailand. As one direction, the plan is to establish a venture from KKU with a governmental support, such as NEDO-NIA's startup business of a Thai venture.

7. Scientific Achievements and Implemented Activities (Publication, Research Exchange, Workshop, etc.)

For this item, please fill in the attached Excel file.

All Scientific Achievements and Implemented Activities are listed in the attached file “e-asia_Scientific Achievements_Form_V1.0-Narimatsu.xlsx”.

8. Recommendations and Comments to the Program

We thank very much for the various supports from the e-Asia program such as opportunities for training of young researchers and fellowship for a student visiting Japan in addition to the financial support to implement the research.

9. Others (agenda of workshop, photos of research teams, meetings, and etc.)

Photos of regular meetings

A. Pre-meeting at Kumamoto University July 22, 2015

KKU members have collaborated for many years with Associate Professor Norie Araki, Kumamoto University. AIST and KKU members participated in a pre-meeting for this project at Araki's lab.

B. Kickoff meeting at AIST November 11, 2015

All members gathered at AIST and discussed in detail the future joint research. Some of them moved to Miyagi (Matsushima) and participated in the 6th ACGG meeting.

C. First annual meeting at KKU and UHS February 9, 2016

Khon Kaen University, Thailand

University of Health Sciences (Lao)

D. Second annual meeting at Kobe November 25, 2016

Group photo and tea ceremony

E. Technical training at AIST for young researchers from KKU
(2 weeks in March 2017)

During their stay for two weeks, research meeting was opened in addition to the training course.

F. Third annual meeting at KKU January 2018

e-Asia symposium at KKU
e-Asia symposium on ”Current Glycosciences and Glycotechnology: Application for Medicine” 2018/1/5
Agenda:

・Welcome address by Prof. Sopit Wongkham & Prof. Hisashi Narimatsu

・About e-Asia JRP: Mr. Toru Sano, Japan Agency for Medical Research and Development (AMED)

・ “Summary of e-Asia Joint Research Program achievements and future perspective of collaborative research in Asia” Prof. Hisashi Narimatsu, AIST/GL-i, Japan
・ “Technologies and databases for glycosciences in AIST, JAPAN” Dr. Kiyohiko Angata, GL-i /AIST, Japan
・ “Differential glycan profiling with lectin microarray for cholangiocarcinoma-specific glyco-alteration discovery” Dr. Atsushi Matsuda, Keio University, Japan
・ “Multi-lectin assay system: from the basics to the practical use” Dr. Asushi Kuno, GL-i /AIST, Japan
・ “Glycosphingolipids of cholangiocarcinoma” Asst. Prof. Krajang Talabnitn, Suranaree University of Technology, Thailand
・ “Intraductal papillary Vor Luvira, MD., Khon Kaen University, Thailand
・ “Serum Wisteria floribunda agglutinin-positive sialylated mucin 1 as a marker of progenitor/biliary features in hepatocellular carcinoma” Dr. Namiki Izumi, Japanese Red Cross Musashino Hospital, Japan
・ “Molecular epidemiological study on liver fluke infection-related cholangiocarcinoma in Thailand” Prof. Masanao Miwa, Nagahama Institute of Bio-Science and Technology, Japan
・ “Integrated proteo-glyco-genomics: Identification of the potential clinical target of cancer stem cells” Prof. Norie Araki, Kumamoto University, Japan
・ “Increasing of GalNAc-associated glycan in cholangiocarcinoma detected by Vicia villosa lectin” Marutpong Detarya, Khon Kaen University, Thailand
・ “Immunohistochemical analyses of O-GlcNAcylation and inflammasomes via NF-κB signaling pathway in oral mucosa from oral lichen planus patients” Do Thi Thao, Khon Kaen University, Thailand

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)

0 Total

1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Matsuda A, Kuno A, Nakagawa T, Ikehara Y, Irimura T, Yamamoto M, Nakanuma Y, Miyoshi E, Nakamori S, Nakanishi H, Viwatthanasittiphong C, Srivatanakul P, Miwa M, Shoda JI, Narimatsu H. Lectin microarray-based sero-biomarker verification targeting aberrant O-linked glycosylation on mucin 1. Anal Chem. 2015 Jul 21;87(14):7274-81.	doi: 10.1021/acs.analchem.5b01329	published		Japan
Yamaguchi T, Yokoyama Y, Ebata T, Matsuda A, Kuno A, Ikehara Y, Shoda J, Narimatsu H, Nagino M. Verification of WFA-Sialylated MUC1 as a Sensitive Biliary Biomarker for Human Biliary Tract Cancer. Ann Surg Oncol. 2016 Feb;23(2):671-7.	doi: 10.1245/s10434-015-4878-4	published		Japan
Shoda J, Matsuda A, Shida T, Yamamoto M, Nagino M, Tsuyuguchi T, Yasaka T, Tazuma S, Uchiyama K, Unno M, Ohkohchi N, Nakanuma Y, Kuno A, Narimatsu H. Wisteria floribunda agglutinin-sialylated mucin core polypeptide 1 is a sensitive biomarker for biliary tract carcinoma and intrahepatic cholangiocarcinoma: a multicenter study. J Gastroenterol. 2017 Feb;52(2):218-228.	doi: 10.1007/s00535-016-1230-0	published		Japan
Tamaki N, Kuno A, Matsuda A, Tsujikawa H, Yamazaki K, Yasui Y, Tsuchiya K, Nakanishi H, Itakura J, Korenaga M, Mizokami M, Kurosaki M, Sakamoto M, Narimatsu H, Izumi N. Serum Wisteria Floribunda Agglutinin-Positive Sialylated Mucin 1 as a Marker of Progenitor/Biliary Features in Hepatocellular Carcinoma. Sci Rep. 2017 Mar 21;7(1):244.	doi: 10.1038/s41598-017-00357-8	published		Japan
Matsuda A, Higashi M, Nakagawa T, Yokoyama S, Kuno A, Yonezawa S, Narimatsu H. Assessment of tumor characteristics based on glycoform analysis of membrane-tethered MUC1. Lab Invest. 2017 Sep;97(9):1103-1113.	doi: 10.1038/labinvest.2017.53	published		Japan

5 Total

Lists of Achievements and Implemented Activities

2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
October 14, 2016	Oral Presentation	Atsushi Kuno, "Development of a new diagnostic system against liver fluke infection under the efforts of THA/LAO/JPN researchers in the e-ASIA Joint Research Program", 8th ACGG Meeting, Wuxi
December 17-20, 2017	Oral Presentation	Atit Silsirivanit, "The e-Asia Joint Research Program (JPN/LAO/THA): Comparative analysis of serum markers for detection of cholangiocarcinoma in Thai and Japanese", 9th ACGG Meeting, Hongkong

2 Total

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
July 23-24, 2015	Guest/Invited Speaker	Sopit Wongkham, "High glucose enhances aggressive phenotypes of cholangiocarcinoma",13th JHUPO Kumamoto, Japan	Thailand
August 13-14, 2015	Guest/Invited Speaker	Sopit Wongkham, "Liver fluke associated cholangiocarcinoma:cA silent killer of Southeast Asia", eAsia workshop. Yangon, Myanmar	Thailand
September 28, 2015	Poster Session	Atsushi Matsuda, "An effective method for exploring tumor-characteristic glycoforms of cell surface mucins", HUPO 14th Annual World Congress, Vancouver	Japan
November 13, 2015	Poster Session	Atsushi Matsuda, "Glycan profiling of cell surface mucin from tissue sections", 7th Asian Community of Glycoscience and Glycotechnology, Matsushima	Japan
August 24, 2016	Guest/Invited Speaker	Atsushi Kuno, "Multilectin assay for glycobiomarker development", Warren Workshop 2016, Sapporo	Japan
September 22, 2016	Guest/Invited Speaker	Atsushi Kuno, "Clinical Demand-oriented Lectin Microarray Analysis", HUPO 2016 Post-Congress Glyco-Mini Symposium, Taipei	Japan
October 13-16, 2016	Guest/Invited Speaker	Sopit Wongkham, "Membranous High Mannose N-glycans Associate with Metastasis of Cholangiocarcinoma Cells" The 8th Asian Community of Glycosciences and Glycotechnology Conference, National Chengkung University, Wuxi, Chaina	Thailand
February 7-9, 2017	Guest/Invited Speaker	Sopit Wongkham, "Multidisciplinary approaches for discovery cholangiocarcinoma biomarkers" US-Japan Cooperative Medical Sciences Program 19th International Conference on Emerging Infectious Diseases in the Pacific Rim. Seoul, Korea.	Thailand
July 24, 2017	Guest/Invited Speaker	Atsushi Kuno,"Lectin microarray-based glycobiomarker development targeting O-glycosylated proteins", 14th International Workshop on Carcinoma-associated Mucins, Cambridge	Japan
July 24, 2017	Poster Session	Atsushi Matsuda," Differential glycan profiling of membrane-tethered MUC1 for exploring tumor-characteristic glycoforms", 14th International Workshop on Carcinoma-associated Mucins, Cambridge	Japan
September 19, 2017	Poster Session	Atsushi Matsuda," Glycan profiling for exploring relationship of MUC1 glycoform with tumor-characteristics by lectin microarray", 16th Human Proteome Organization (HUPO) World Congres, Dublin	Japan
December 17-20, 2017	Oral Presentation	Atsushi Matsuda," Comprehensive glycoform analysis of endogenous MUC1 for disease-specific glycol-alteration discovery.", 9th ACGG Meeting, Hongkong	Japan
November 18-21, 2018	Guest/Invited Speaker	Atit Silsirivanit, "Integrated glycobiomarker analysis for diagnosis of cholangiocarcinoma", The 10th Asian Community of Glycosciences and Glycotechnology Conference, National Chengkung University, Tainan, Taiwan	Thailand

13 Total

Lists of Achievements and Implemented Activities

3. Workshops, Seminars, Symposia and Other Events (Organized by the Project)

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
5-Jan-18	Sopit Wongkham	Current Glycosciences and Glycotechnology: Application for Medicine	Thailand, Khon Kaen, Faculty of Medicine, Khon Kaen University	60	

1 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination (Country, City, Research Organization etc)	Description of Exchange Content/Purpose	Duration of Exchange (autocompleted)
January 6, 2016	January 16, 2016	Taro Yamada	Japan	Yamada University	Professor	NANOTEC,NECTEC, Bangkok	○○	11
January 11, 2016	January 16, 2016	Atit Silsirivanit	Thailand / Japan	Khon Kaen University / Kumamoto University	Lecturer	AIST, Japan	Training on WFA-MUC1 analysis method	6
March 12, 2017	March 25, 2017	Atit Silsirivanit	Thailand	Khon Kaen University	Assistant Professor	AIST, Japan	Training on Antibody class switching	14
March 12, 2017	March 25, 2017	Kanlayanee	Thailand	Khon Kaen University	Assistant Professor	AIST, Japan	Training on Antibody class switching	14
March 12, 2017	March 25, 2017	Wunchana Seubwai	Thailand	Khon Kaen University	Assistant Professor	AIST, Japan	Training on Antibody class switching	14
								0

Total (Person) 4 Total (Persond-day) 48

Lists of Achievements and Implemented Activities

5. Patent Applications

5. 1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team

0 Total (Number of Application)

0 Total (Number of Registration)

5. 2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)

0 Total (Number of Application)

0 Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team

0 Total