As of Mar 19, 2015

(Abroad・Domestic) Internship report form (Student) 2016/12/30(Year/Month/Day)

Name	Yogendra Shah
Laboratory	Division of Bioresources (Research Center for Zoonosis Control)
Year (Grade)	Four (D4)
Internship institution	German Nepal Tuberculosis Project (GENETUP), Kalimati, Kathmandu, Nepal GENETUP Hospital, Kalimati, Kathmandu, Nepal
Internship period	Internship period: 11/04/2016 - 12/19/2016 (Departure Date from Sapporo:11/04/2016, Arrival Date in Sapporo: 12/20/2016)
Purpose	To learn and gain skills on different conventional laboratory techniques applied in diagnosis of tuberculosis bacteria at GENETUP. Also, explore future research career path in the field of tuberculosis and infectious diseases in Nepal.

Internship activity at GENETUP and different research institutes in Nepal

- The reason why you chose this institute

GENETUP is one of the oldest institutes with highly equipped laboratory facilities in Nepal. It is well known especially for working in the field of tuberculosis (TB) in Nepal and contributing to the control of the disease by providing diagnostics facilities for the last several decades. Another reason is to know the actual situation of TB in Nepal. Since GENETUP is a major player I am interested in working for above reason in addition to further carry out the TB research to enhance my current study. In addition to these, our divisions have a strong collaboration with GENETUP for nine years.

- Result of the activity (about 800 words、provide photos, tables and figures that clearly show the activities during the period)

The main objective of the internship activity was to learn the skills on different conventional laboratory techniques for diagnosis of TB disease and also explore future research career in the field of TB and other infectious diseases in Nepal. I have briefly summarized the activities of my internship activity as follows:

1. Learning skills of conventional laboratory techniques

Before conducting the experiments, the person of the Laboratory In charge, Mr. Bhagwan Maharjan, arranged observation training with each conventional laboratory procedures applied for TB diagnosis at GENETUP. I also performed the following experiments mainly; fluorescence microscopy staining, culture on LJ media, Xpert MTB/RIF assay, molecular line probe assays (first and second line drugs), phenotypic drug susceptibility testing (DST), color test DST, media preparation for DST, nitrate reduction test and DNA extraction according to their standard operating procedures (SOP) at GENETUP (Photo 1). Besides the routine experiments, our laboratory has also supported introduction and establishment of some molecular genotyping methods at Healthy Nepal like

Dry LAMP, Wet LAMP, Spoligotyping, 15 loci mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) and electrophoresis of MIRU-VNTR typing (Figure 1).

Photo 1: Performing conventional routine techniques at GENETUP

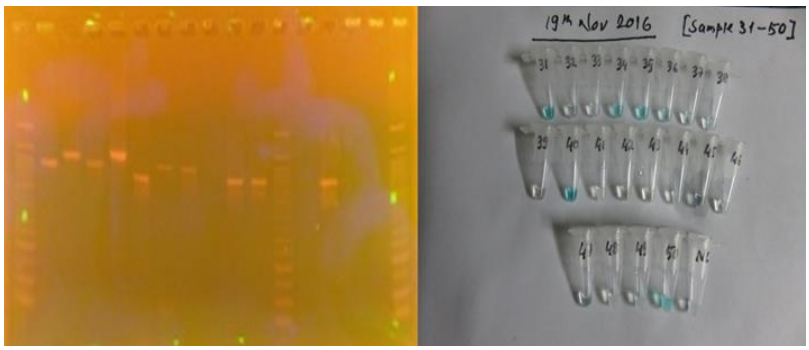

Figure 1: MIRU-VNTR typing electrophoresis picture and Dry LAMP

2. Exploring research career at different Institutes/ Hospitals in Nepal

The second objective of internship was to explore future research career opportunities in the field of TB and other infectious disease at different institutes/hospitals as listed below (Photo 2 and Photo 3). I had an opportunity to discuss with head officials about future career and job opportunities as a researcher in their institutes in Nepal after I graduated. I also observed the laboratory facilities and running projects activities at respective organization. According to the organization's head, they were a quite positive response and I gathered important information for getting post as a researcher in their organization. During the exploring research career, I was able to meet the top ranking researchers and academic officers this experience will be helpful for further setup a milestone in my career. In addition, I now have a better understanding about the actual situation of TB

and other infectious diseases in Nepal through their research progress. However, in my experience, I found a big difference in terms of laboratory facilities setting between Japan and Nepal (a poor resource setting country).

- German Nepal Tuberculosis Project (GENETUP)
- Chest Hospital (Nepal Anti-tuberculosis Association)
- Healthy Nepal
- Leprosy Control Division, Department of Health Services, Ministry of Health, Kathmandu, Nepal
- National Zoonoses and Food Hygiene Research Center (NZFHRC)
- Sukraraj Tropical and Infectious Disease Hospital (STIDH)
- Nepal Academy of Science and Technology (NAST)
- National Forensic Science Laboratory
- National Tuberculosis Center (NTC)
- Anandaban Hospital, Patan Clinic
- Anandaban Hospital, Lele, Lalitpur
- Walter Reed/ AFRIMS Research Unit Nepal (WARUN)
- Microbiological Research Organization Nepal (MIRON)
- Mahakali Zonal Hospital (MZH)
- Farwestern University

Photo 2 and 3: Visited and observed laboratory facilities at different institutes/hospitals in Nepal

3. Attending seminar delivered by Dr. Sujan Shresta

I attended a seminar organized by Nepal Academy of Science and Technology (NAST) in title “Dengue and Zika pathogenicity and Immunology". In this seminar, a presentation delivered by Dr. Sujan Shresta (Associate Professor from LaJolla Institue for allergy and immunology) mainly focused on development of rapid diagnostic kits by performing the *invitro/invivo* methods for Zika and Dengue virus. I had opportunity to explore and discuss with top ranking senior scientific officers about future career opportunities in the field of science and technology as well as job possibilities as researcher at NAST.

Photo 4: Dr. Sujan Shresta delivering presentation at NAST

Photo 5: Senior scientific officer at NAST

4. Learning skills to design the Standard Operating Procedure (SOP)

I learned the skills to design the SOP for laboratory diagnosis for TB at GENETUP. Mr. Bhagwan Mahrajan and Mr. Bijendra Ray were the two responsible persons for my training on how to design the SOP to perform TB diagnosis provided by Gauting Supra National reference Laboratory, Germany. They kindly provided me a training class on how to design the good SOP for each conventional methods and quality control for reagents preparation. After that, as an assignment I designed an SOP for AFB staining fluorescence method based on the available resources setting according to their institute. I also performed the quality control for freshly prepared reagents i.e. fluorescence staining microscopy and other equipment’s used at GENETUP.

- What do you think the positive impact of the activity will have on your further career path?

This internship activity has provided an opportunity to learn and gain skills of conventional routine techniques for diagnosis of TB bacteria at GENETUP. I also learn how they treat their TB infected patients and observed what kind of laboratory techniques they applied in diagnosis of TB in their respective hospitals. During this internship, I got opportunity to visit and explore the different research institutes or hospitals and actively interact

with officials involved in TB and other infectious diseases work about the future career possibilities in different research areas and job opportunities at their institutes. Therefore, I think that this internship has increased the chances of getting a research position in some reputed research institutes or hospitals in my home country after graduation. This internship activity will also help to get acquainted with TB prevention activities in Nepal and identifying future research areas.

- Advice for your junior fellows

I would like to advice to junior fellows, to firstly select the potential interested research areas and institute that will be help to determine the future career path as a researchers after graduation. The most important thing to remember is to sketch the long term career goal before starting any crucial tasks in the life in this case i.e. internship activity. I also want to suggest that junior fellows start the internship preparation at least three months early from the tentative date of visit to respective the country or institute. Before travelling please have a look over/check the required documents needed for respective institute or country and arrange them properly to avoid trouble later. At last, try your best, be hardworking, enjoy the trip and learn productive styles and methods that can be easily transferred to your laboratory.

Table 1: Outline activities of Internship and Field epidemiology study at GENETUP and different research institutes in Nepal.

Date	Activities
November 4, 2016:	-Departure to Nepal for Internship and Field epidemiology study, Arrive Kathmandu, Nepal
November 5, 2016	-Discussed with Mr Bhagwan sir about the internship and field epidemiology study
November 6, 2016	-Discussed with Director Dr Bhawana Shrestha about the brief purpose my internsip and field epidemiology study,-Submitted proposal to Director Dr Bhawana Shrestha and official documentation (Bench fee) at GENETUP
November 7, 2016	- Discussed with Dr Pandey about internship and field epidemiology study -Observed the routine laboratory work for diagnosis of tuberculosis at GENETUP (MDR plus assay procedure)
November 8, 2016	- Observed the routine laboratory work for diagnosis of tuberculosis at GENETUP (Genexpert),-Observed Dry LAMP and Wet LAMP performed experiment by Jeewan San
November 9, 2016	- Observed the samples collection procedure and routine laboratory work for diagnosis of tuberculosis at GENETUP (Fluorescence microscopy and monthly maintenance and cleaning (0 5% sodium hypochlorite solutionof Gene-xpert machine) at GENETUP,-Prepared the reagents and performed the MIRU-VNTR experiment locus 1 and locus 2
November 10, 2016	- Observed of the equipment and performed the Gene-xpert, fluorescence microscopy staining and culture on LJ media for diagnosis of TB bacteria
November 11, 2016	-Performed the fluorescence microscopy staining, culture on LJ media and Gene-xpert for diagnosis of TB bacteria,-Observed the Dry LAMP performed experiment by Jeewan San
November 13, 2016	- Performed the fluorescence microscopy staining, culture on LJ media and observed the staining slides for diagnosis of TB bacteria at GENETUP ,-Discussed with clinical research unit head Dr Sher Bahadur Pun about the laboratory techniques applied for diagnosis of TB (x-ray and microscopy available) at Sukraraj Tropical and Infectious Disease Hospital
November 14, 2016	-Performed the fluorescence microscopy, culture on LJ media and Gene-xpert for diagnosis TB bacteria at GENETUP,-Visited the Nepal anti-tuberculosis association hospital and observed the diagnostic facilities related to TB
November 15-16, 2016	-Performed the fluorescence microscopy, culture on LJ media, Gene-xpert and phenotypic DST for diagnosis of TB bacteria at GENETUP,-Visited the NATA chest hospital, -Observed the dry LAMP and wet LAMP experiment performed by Jeewan San at Healthy Nepal
November 17, 2016	-Performed the fluorescence microscopy, culture on LJ media, Gene-xpert and Genotype MTBDRplus line probe assay (first line anti-TB drugs) at GENETUP, - Observed the dry LAMP and wet LAMP experiment performed by Bhagwan Sir at Healthy Nepal.
November 18, 2016	-Performed the fluorescence microscopy, Gene-xpert and observed culture on LJ media, nitrate reduction test, Genotype MTBDRplus line probe assay (second line anti-TB drugs) at GENETUP -Performed the electrophoresis of MIRU-VNTR typing for locus 1 and locus 2 ,- Observed the dry LAMP experiment performed by Bhagwan sir at Healthy Nepal
November 19, 2016	- Observed the dry LAMP and wet LAMP experiment performed by Bhagwan sir and master's thesis student at Healthy Nepal
November 20, 2016	-Performed the fluorescence microscopy staining, culture on LJ media and observed Gene-xpert, phenotypic DST at GENETUP, -Visited to Leprosy Control Division at Teku and discussed with Dr Pandey sir about future career in the field of TB research and opportunity in Nepal
November 21, 2016	- Performed the fluorescence microscopy staining and culture on LJ media GENETUP, -Visited National Zoonoses and Food Hygiene Research Center (NZFHRC) at Tahachal,-Discussed with project coordinator Ms Minu Sharma about current project as future career in the field of zoonosis research and job opportunity at NZFHRC
November 22-23,2016	-Performed the fluorescence microscopy staining and culture on LJ media Gene-xpert and observed Genotype MTBDRplus line probe assay (second line anti-TB drugs), color test DST at GENETUP -Visited DOTS/MDR-TB clinic and observed treatment regimen for MDR-TB/pre-XDR TB at NATA
November 24, 2016	-Performed the fluorescence microscopy staining, culture on LJ media, Gene-xpert and observed color test DST at GENETUP,-Visited to Microbiological research organization Nepal (MIRON) and discussed with laboratory head about current running project and job opportunity

November 25 , 2016	- Performed the fluorescence microscopy staining, culture on LJ media, Gene-xpert and observed Genotype MTBDRplus line probe assay (second line anti-TB drugs) at GENETUP, -Attended the seminar organized by Japanese Universities Alumni Association, Nepal (JUAAN) in title "A Journey in Harmony: 60 years of Nepal-Japan Relations" at Hotel Himalayan
November 27, 2016	- Attended the seminar organized by Nepal Academy of Science and Technology (NAST) in title "Dengue and Zika pathogenicity and Immunology" - Discussed with top ranking senior scientific officers about future career in the field of science and technology as well as opportunity of job at NAST - Observed the National Forensic Science Laboratory at Khumaltar and discussed with senior scientific officers about the future career in the field of forensic science and opportunity of job
November 28, 2016	- Observed the laboratory techniques applied for diagnosis of TB at National Tuberculosis Center (NTC), -Discussed with senior medical technologist (Microbiologist) Mr Gokarna Raj Ghimire regarding the future career in the field of TB research and opportunity of job at government sector organization in Nepal like NTC
November 29, 2016	-Observed the laboratory facilities applied for diagnosis of different neglected tropical infectious diseases and ART clinic including TB at Sukraraj Tropical and Infectious Disease Hospital (STIDH),-Discussed with Director Dr Ramesh Kumar Kharel and laboratory head about the current running projects and future career in the field of tropical infectious diseases at STIDH
November 30, 2016	-Observed the basic laboratory work for diagnosis of leprosy disease at Anandaban Hospital, Patan clinic,- Visited and observed laboratory facilities at Walter Reed/AFRIMS Research Unit Nepal (WARUN), -Discussed with Head of Station Dr Sanjaya Kumar Shrestha and laboratory head Mr BishnuRayamajhi about the current running projects as well as future career in different research areas and job opportunity at WARUN, - Visited to NZFHRC and discussed with Microbiologist Mr Dhan Kumar Pant regarding the previous work on Japanese encephalitis virus documentation.

December 01, 2016	-Visited and observed the routine laboratory techniques applied for diagnosis of leprosy disease at Anandaban Hospital and Clinic Lab, Lele, Lalitpur -Discussed with Laboratory Head Dr Deanna A Hagge about the current projects on leprosy disease as well as future career in mycobacterial research laboratory at Anandaban Hospital - Observed laboratory facilities at Microbiological Research Organization Nepal (MIRON),-Discussed with Mr PrabinShakya regarding the future career in multidisciplinary research areas and also future plan to apply grants for zoonosis disease research in context of Nepal
December 02, 2016	-Observed and performed the routine laboratory techniques applied for diagnosis of TB bacteria at GENETUP -Performed the MIRU-VNTR typing for locus 1, locus 2, locus 3 and locus 4 -Observed the Dry LAMP experiment performed by Bhagwan sir at Healthy Nepal
December 04,2016	- Performed and observed the fluorescence microscopy staining, Culture on LJ media, Gene-xpert and DNA extraction at GENETUP, -Prepared the agarose gel and performed the electrophoresis of PCR products (MIRU-VNTR locus)
December 05, 2016	-Performed the fluorescence microscopy staining, Culture on LJ media and Gene-xpert for diagnosis of TB bacteria at GENETUP,-Performed the MIRU-VNTR typing for locus 5, locus 6 and locus 7 at Healthy Nepal
December 06, 2016	-Performed the conventional routine techniques i e (fluorescence microscopy staining, culture on LJ media and Gene-xpert) at GENETUP, -Performed the MIRU-VNTR typing for locus 8, locus 9, locus 11, locus 12, locus 13, locus 14, locus 15 and locus 21 at Healthy Nepal
December 07-8, 2016	-Performed the conventional routine techniques i e fluorescence microscopy staining, culture on LJ media, Gene-xpert and observed color test DST at GENETUP, -prepared the agraose gel and performed the electrophoresis of PCR products (MIRU-VNTR locus),-Observed the Dry LAMP experiment performed by master's thesis student at Healthy Nepal
December 09-10, 2016	-Performed and observed the conventional routine techniques i e fluorescence microscopy staining, culture on LJ media, Gene-xpert and reagents preparation for DST at GENETUP, -Prepared the agraose gel and performed the MIRU-VNTR typing for locus 1, locus 2, locus 3, locus 4 and electrophoresis of PCR products (MIRU-VNTR locus) at Healthy Nepal
December 11, 2016	-Performed and observed the conventional routine techniques i e fluorescence microscopy staining, culture on LJ media, Gene-xpert and media preparation for DST at GENETUP, -observed the Wet LAMP experiment performed by master's thesis student at Healthy Nepal
December 12/13, 2016	-Performed and observed the conventional routine techniques i e fluorescence microscopy staining, culture on LJ media and Gene-xpert at GENETUP
December 14, 2016	-Visited and observed the laboratory techniques applied for diagnosis of TB and other diseases at Mahakali Zonal Hospital (MZH), -Discussed with Hospital Head Dr Shree Hari Dutta Bhatta about the current laboratory facilities for diagnosis of TB and, - Explore the future career path in the field of infectious diseases at MZH
December 15, 2016	- Visited and observed the different faculty laboratory at Farwestern University (FWU),-Discussed with Science and Technology Co-ordinator Mr Madan Singh Bohara regarding the new update information about university, -Explore the future career as well as job opportunity (teaching assistant) at FWU
December 16, 2016	-Performed and observed the conventional routine techniques i e fluorescence microscopy staining, culture on LJ media, Gene-xpert and media preparation for DST at GENETUP
December 18, 2016	- Performed and observed the conventional routine techniques i e fluorescence microscopy staining, Culture on LJ media and color test DST for diagnosis of TB bacteria at GENETUP,- Discussed with Dr Ajay Poudel regarding the new update information about the future career and job opportunity as teaching assistant at Medical Colleges in Nepal
December 19, 2016	- Observed the conventional routine techniques for diagnosis of TB bacteria at GENETUP, -Discussed with Mr Bhagwan sir regarding the final progress report about the internship and field epidemiology study during stay at GENETUP, -Help to design the standard operating procedure (SOP) for fluorescence microscopy staining. - Departure to Sapporo, Japan
December 20, 2016	- Arrived to Sapporo, Japan

Field Epidemiological Study at GENETUP

Background

TB caused by MTB is a major public health problem in Nepal. Despite the successful TB control program in Nepal, the number of TB cases has not decreased in the last decade. The numbers of multi-drug resistant TB (MDR-TB) cases are increasing every year in Nepal and the reason behind this phenomenon is still unknown. Therefore, comprehensive studies on MTB transmission in Nepal are needed to address the increasing pattern of MDR-TB cases.

Objectives

The main aim of this study was to better understand the molecular epidemiological features and transmission dynamics of MDR-TB in Nepalese TB patients and to evaluate the pre and post-earthquake effect on TB patients in Nepal by performing conventional routine methods at GENETUP.

Major activities of field epidemiological study

I learned the skills of sputum collection procedure, processing, handling, decontamination, digestion, media preparation, reagent's preparation, instruments operating, documentation and reporting about the TB disease at GENETUP. During the field epidemiological study, I collected a total of 225 sputum samples of suspected cases with demographic information and carried out conventional methods at GENETUP. Results are summarized in table below.

Treatment		Fluorescence microscopy results	
New patient	143	Positive	28
		Negative	115
Retreatment	82	Positive	20
		Negative	62
Total	225	Total	225

Results were analyzed for comparison between predominant lineages of isolates and trends of increasing MDR-TB among patients. The information will be useful in further study analysis in tracing outbreak isolates, source of transmission and identification of novel SNPs. It will also be useful to evaluating MDR-TB trends in pre and post-earthquake transmission. Additionally, the knowledge from this study could help in disease surveillance and strengthening of control measure of MDR-TB in Nepal.

Due to limited time I did not have a chance to collect mycobacterial culture positive samples. Later, I will follow up on sample collection and DNA extraction of TB isolates with laboratory in charge, Mr. Bhagwan Maharjan. The extracted bacterial DNA will be transported to Japan for further analysis with approval from Nepal Health Research Council from Nepal government body. Further, molecular analysis such as Spoligotyping, 12 loci Mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR), Single nucleotide polymorphism (SNP), DNA sequencing typing and whole genome sequencing analysis (WGS) will be performed at Division of Bioresources, Hokkaido University Research Center for Zoonosis Control to better understand the molecular epidemiological features as well as pre and post-earthquake effects among Nepalese TB patients.

Photo 6: Performing smear preparation, observing fluorescent microscopy staining slide and inoculating AFB bacilli in LJ media

As of Mar 19, 2015

Summary of my Internship/Field epidemiological activity in Nepal

I returned back to Japan from Nepal (Internship/Field epidemiological study) on December 19 and arrived on December 20, 2016. This internship/field epidemiological study was very fruitful in learning many important skills with specific knowledge about the different conventional routine techniques to diagnosis TB in Nepal that will surely help me to identify the future career as a researcher after graduation. I would like to sincerely thank to leading office especially Prof. Motohiro Horiuchi, Yuki Maki San, Terashima San and leading steering committee for providing opportunity to conduct internship/field epidemiological study in Nepal. I would also sincerely thank my supervisors Prof. Yasuhiko Suzuki and Prof. Chie Nakajima sensei for giving me this big opportunity to conduct the internship/field epidemiology and guiding in my study. Finally, I would like to sincerely thank Ms. Bhawana Shrestha and Mr. Bhagwan Maharjan for accepting me as an intern student and guiding me during my study period at GENETUP laboratory.

Approval of supervisor	Institution・Official title・Name Division of Bioresources Research Center for Zoonosis Control 印 Hokkaido University Professor Yasuhiko Suzuki

※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.

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