Project Completion Report

Surveillance and Laboratory Support for Emerging Pathogens of Public Health Importance in Ghana

March 2022

Japan International Cooperation Agency

JICA

The University of Tokyo

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JR
22-057

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ANNEX 3: PDM

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Contents of the Project Completion Report

I. Basic Information of the Project

1. Country

The Republic of Ghana

2. Title of the Project

Surveillance and Laboratory Support for Emerging Pathogens of Public Health Importance in Ghana

3. Duration of the Project (Planned and Actual)

August, 2016 - March, 2022

Because of the global spread of the coronavirus disease 2019 (COVID-19), some of the project activities were unfortunately suspended. Under this unfortunate circumstance, the project duration was extended for 7 months in order to achieve the project purpose.

4. Background (from Record of Discussions(R/D))

The Republic of Ghana has witnessed a record growth in the recent years as it entered the lower-middle income country status in 2010 with an economic growth rate that has constantly been over or close to 10%. The health status of its people, however, has lagged behind where the under-five mortality rate is 60/1000 live births opposed to the MDG goal of 53/1,000 (2014, Ghana DHS) and maternal mortality rate estimated at 380/100,000 births opposed to the MDG goal of 185 (2010, WHO). The country suffers from a high burden of infectious diseases, such as malaria, acute respiratory illness, HIV/AIDS, and diarrheal diseases, as well as an increase of non-communicable diseases in the recent years. Furthermore, while the country suffered from a cholera outbreak in 2014, three countries in the West African sub-region were ravaged by the Ebola Disease Virus (EVD) outbreak. Ghana remains to be under threat of emerging and re-emerging infectious diseases.

5. Overall Goal and Project Purpose (from Record of Discussions(R/D))

This Project aims to strengthen the surveillance and response system by establishing a model for basic research-linked surveillance system in Ghana and

deepening the understanding of the protection mechanism(s) of intestinal mucosal tissue and its immunity to enteric infectious diseases such as cholera and the role of host immune responses to HIV.

6. Implementing Agency

The Project was implemented through collaborative research with Japanese research institutions including the Institution of Medical Science, the University of Tokyo (IMSUT), Faculty of Medicine, Mie University (MU), National Mie Hospital (NMH), and National Institute of Infectious Diseases (NIID), and Ghanaian institutions including Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana (NMIMR) and Ghana Health Service (GHS). IMSUT and NMIMR led the SATREPS project as the representative institution from Japan and Ghana, respectively.

II. Results of the Project

1. Results of the Project

1-1 Input by the Japanese side

(1) Expert dispatch: the derail are listed on Annex1-1 Dispatched Experts

Chief Advisor (Short-term expert)

2016 JFY:　1 dispatch (9/25-9/26)
2017 JFY:　3 times dispatches (8/26-30, 11/16-20, 2/27-3/2)
2018 JFY:　0 time
2019 JFY:　1 time (11/25-12/1)

Surveillance (Long/short-term expert)

2016 JFY:　2 dispatches (long term experts)
2017 JFY:　3 dispatches (short term experts)
2018 JFY:　4 dispatches (short term experts)
2019 JFY:　11 dispatches (short term experts)
2020 JFY:　0 dispatch
2021 JFY:　1 dispatch (short term expert)

Microbiome Analysis (Long/short-term expert)

2016 JFY:　1 dispatch (short term expert)
2017 JFY:　1 dispatch (short term expert)
2018 JFY:　0 dispatch
2019 JFY:　0 dispatch
2020 JFY:　0 dispatch

2021 JFY:　0 dispatch

Experts for immunology, virology, infectious diseases and epidemiology

(Short-term experts)

2016 JFY:　5 dispatches (short term experts)
2017 JFY:　6 dispatches (short term experts)
2018 JFY:　6 dispatches (short term experts)
2019 JFY:　2 dispatches (short term experts)
2020 JFY:　1 dispatch (short term expert)
2021 JFY:　2 dispatches (short term experts)

Project Coordinator (Long-term expert)

2016 JFY:　1 dispatch (long term expert)
2017 JFY:　1 dispatch (long term expert)
2018 JFY:　1 dispatch (long term expert)
2019 JFY:　2 dispatches (long term experts)
2020 JFY:　1 dispatch (long term expert)
2021 JFY:　2 dispatches (long term expert and short term expert)

(2) Receipt of training participants in Japan: see attached 1-4 List of technical transferring training (in Japanese).

Strengthening Surveillance,

2017	5 dispatches
2018	1 dispatch
2019	3 dispatches
- Participation of Symposium, January 2017	3 dispatches

(3) Meetings:

- JCC　3 times (2018, 2019, 2021): Annex 4 Minutes of JCC
- International Symposium,
  - ・ September 2016, Kick-off Meeting and Scientific Symposium “Project for Surveillance and Laboratory Support for Emerging Pathogens”
  - ・ June 2018, Noguchi Hideyo Memorial Museum Symposium on Infection Disease and Immunity on the occasion of the formal presentation ceremony of Dr. Hideyo Noguchi’s autopsy record from Ghana to Japan
  - ・ November 2019, International Conference “Sustainable Global

Health Gains through Partnerships on Biomedical research" under NMIMR 40th anniversary.

- November 2021, Scientific Symposium on SATREPS Project

(4) Equipment Provision:　see attached Annex 1

(5) Overseas activities cost: JY131,907,000-

1-2 Input by the Ghana side: see attached 1-2. List of Counterparts

（1） Counterpart assignment:

- Prof. Dorothy Yeboah-Manu, Director of Noguchi Memorial Institute for Medical Research (NMIMR), Project Director from 31st July 2022.
- Dr. Asiedu-Bekoe, Director, Public Health Div., Ghana Health Service (GHS)
- Dr. Dennis Laryea, Acting Head, Disease Surveillance Dep, GHS, Project Manager, from 1st of January 2021
- Dr. John Kofi Odoom, Head, Virology, NMIMR, Project Manager from 1st of August 2020
- Dr. Anthony Ablordey, Bacteriology, NMIMR
- Dr. Margaretta Gloria Chandi, District Director, Ga West Municipal
- Dr. Evelyn Yayra Bonney, Virology, NMIMR
- Dr Adwoa Asante-Poku, Bacteriology, NMIMR
- Dr Ivy Mensah, Bacteriology, NMIMR

（2） Provision of offices: As planned, the Ghana side provided the project office at NMIMR and facility utility, Internet, other personnel cost.

1-3 Activities (Planned and Actual)

The project has implemented the following activities in the PDM and PO (Version 2.0). The project accomplishes almost all of its expected outputs by the end of the project period. The results of outputs are as follows:

Output 1: A sustainable, basic research-linked surveillance for events of public health importance is established.
Activities: 1-1. Establishment of the sentinel surveillance system for diarrhea diseases. 1-1-1 To conduct a baseline survey with developed tools in a selected model site. 1-1-2 To assign 5 medical facilities (target facilities of the Project) as the

sentinel sites of the surveillance of diarrhea diseases in the model site on the basis of the baseline survey.

1-1-3 To provide the medical professionals and other eligible staff with trainings for the introduction and operation of the diarrhea sentinel surveillance.

1-1-4 To conduct training on surveillance geared to staff of local medical facilities and community-based surveillance volunteers (CBSVs) for the improvement of infectious disease surveillance in the entire model site, based on the results of the baseline survey.

1-1-5 To develop Standard Operating Procedures (SOPs) for sample collection, data management and the feedback of test results including drug susceptibility of pathogens.

1-1-6 To support the operational management of surveillance including sample collection and transport at communities and health facilities, monitoring & evaluation, and feedback, for the early detection of IDRS priority diseases.

1-1-7 To transfer the detection techniques of major causative agents of viral diarrhea such as rotavirus, norovirus, etc., as well as important bacteria (as needed basis) from the NMIMR to the NPHRL.

1-2. Development of a prototype of sample banking system linked with the infectious disease surveillance

1-2-1 To construct an information database of clinical samples collected through the passive and active surveillances (the diarrhea surveillance and HIV research, respectively), consist of patient- and pathogen characteristics' including drug susceptibility in the NMIMR.

1-2-2 To arrange storage equipment for the preservation of clinical samples such as blood, feces, etc. as well as isolated and/or extracted DNA, and pathogens, etc. in the NMIMR.

1-2-3 To develop regulations for the operation of a prototype of sample banking system, which stipulates not only the supply/transportation of clinical samples to the NMIMR, preservation, access and withdrawal, but also the maintenance management of the equipment and the information database.

1-3. Functional verification of the basic research-linked surveillance system.

1-3-1 To review the basic research-linked surveillance system from the viewpoints of sustainability and applicability to other regions, followed by

the modification of the system operation including the SOPs. 1-3-2 To verify the operability and effectiveness of the system on the basis of the IDSR performance indicators as well as the implementation of the simulation exercises (if needed), followed by the modification of it as needed basis. 1-3-3 To develop guidelines for the introduction to other regions and operation of the model by packaging the necessary procedures, training materials and its lecturers, operating expenses, timeframe, ledger sheets, etc. 1-3-4 To commence concrete discussions with Ghanaian authorities concerned such as the GHS for the endorsement of the model or the mergence of the elements of it into the current IDSR.
Achievements: Upon the field search and assessment, we selected Ga West as the model area for strengthening of the surveillance and performed base-line survey. The base-line survey was conducted according to the WHO/AFRO guideline for Integrated Diseases Surveillance and Response (IDSR). Based on the assessment, the surveillance system in Ga West was reinforced by performing training courses for health workers, arrangement of sample transfer methods from community to local and National laboratories, and improving pathogen detection and diagnosis test skills at these laboratories. Especially, the accuracy and timely feedback of the results at National Public Health Reference Laboratory (NPHRL) was emphasized and consequently improved. The health center at the model area also launched a weekly epidemiological report on the major infectious diseases for health sectors. A package of surveillance guideline and training system was established in collaboration with Ghana Health Service (GHS) and Noguchi Memorial Institute of Medical Research (NMIMR) to be applied into infectious diseases surveillance in other areas in Ghana. As a first step, a training for analysis and graphic visualization of surveillance data was conducted among health workers in Ga West and Greater Accra, the next target area for surveillance. For accurate pathogen diagnosis, we reinforced a diagnostic system for parasites by replacement of microscopes and staff training, and that for viruses by introducing rapid diagnostic testing. Accordingly, we found that diarrhea attributed to virus infection rather than bacterial infection is unexpectedly predominant in community. Furthermore, the next-generation sequencing (NGS) technique was introduced into NMIMR. Analysis of diarrhea samples in

Ga West using this technique found a group of bacteria that were not previously known to exist in Ghana, such as *Campylobacter* genus. These important findings would greatly contribute to accurate pathogen diagnosis and control of infectious diseases in Ghana.

Further Achievements:

The surveillance at the model area became well-functioned epidemiologically and clinically. NPHRL is also functioning as a reference laboratory with the support of NMIMR. The outcomes from the pilot study will be applied to other areas in Ghana and other countries in Africa. The functional surveillance enables a rapid action for outbreaks of cholera and other emerging pathogens of public health. Since 2021, even under the difficult situation of COVID-19 pandemic, the surveillance system has been introducing at Adabraka polyclinical and Princes Marry Louise Children's Hospital in Greater Accra region. The results from these activities will provide more information for the nationwide approach.

This project proved the importance of viral pathogens in diarrheal diseases in Ghana. This evidence will contribute to the proper use of antibiotics, and mitigate emergence of drug-resistance strains of bacteria. For more precise and extensive screening for viral pathogens, technology transfer is ongoing for scientists visiting at Mie University and Mie National Hospital as a PhD student or a JICA-SDG program trainee in Japan. Continuation of the training of Ghanian doctoral students is an important aspect of the project for the sustainability of the strengthened diarrheal disease system in Ghana.

Furthermore, our new observation for the prevalence of campylobacter provides useful information to clinicians for their treatment strategy of patients with campylobacter infection with the proper gram-staining and clinical manifestations.

Output 2: The capacity of investigation and research for the identification of promoting, resistance and mucosal defense factors as well as the estimation of HIV endemicity status on the basis of genomic analyses of microbiome, HLA, enteric pathogens is strengthened in the NMIMR.

Activities:

2-1. To develop research protocols on HIV and diarrheal diseases in collaboration between Ghanaian and Japanese researchers..

2-2. To provide Ghanaian researchers with the laboratory and classroom

trainings for the analyses of pathogen genome in diarrhea conditions, HLA-associated polymorphisms in Ghanaian HIV carriers, next-generation sequencing of microbiome and bioinformatics for the analyses on the interrelationship of the genomic diversity trilaterally (i.e., microbiome, pathogens and host) .

2-3. To perform the characterization of HIV infection genetically by performing the multiple genomic analyses of HLA-associated polymorphisms and microbiome in Ghanaian HIV carriers with currently available clinical and laboratory data such as CD4+ T-cell counts and viral load as basic information for future development of novel drugs and vaccines.

2-4. To identify promoting, resistance, and/or mucosal defense factors of infections by analyzing the interrelationship of genomic diversity amongst microbiome, pathogens and host HLA, for future risk assessment of infections for susceptibility and severity, the estimation of the response to medications and treatments, and future application of the improvement of infectious controls.

Achievements:

We developed numerous research protocols on HIV and diarrheal diseases in collaboration between Ghanaian and Japanese researchers. We provided multiple training courses in Ghana and Japan for Ghanaian researchers to learn skills for analyses of pathogen genomes in diarrhea conditions and HLA-associated polymorphisms in Ghanaian HIV carriers, next-generation sequencing of microbiome, and bioinformatic analyses. Five Ghanaian students learned molecular biology, microbiology, and immunology including the latest scientific techniques described above at National Institute of Infectious Diseases (NIID) in Japan. Three of them obtained PhD and others are still in the PhD course. All of them has been heavily involved and contributed their expertise for the SATREPS project. They will play a key role for the sustainable and improvement of the joint multi-genomic based research platform between Ghana and Japan.

Polymorphisms in human leukocyte antigen (HLA) are known to have a great impact on infectious disease susceptibility and progression, especially in HIV infection. Prevailing HIV subtypes and HLA genotype distribution are different all over the world, but data on the HIV and HLA interaction have not fully been obtained in West Africa where HIV subtype CRF02_AG is predominant. In this project, HLA class I four-digit allele typing was performed in more than 300 HIV

infected individuals in Ghana by a super high-resolution single-molecule sequence-based typing (SS-SBT) using the next-generation sequencing (NGS) technique. Comparison of the SS-SBT-based data with those obtained by a conventional sequencing-based typing (SBT) revealed incorrect assignment of several alleles by the latter SBT. This project for the first time presented the SS-SBT-based, accurate four-digit typing data on HLA class I alleles in Ghana, West Africa. This enabled us to accurately determine the impact of HLA genotypes on viral load and CD4 count in HIV infection. HLA-associated HIV polymorphisms were also determined.

We then examined the influence of individual HLA alleles on viral loads and CD4 counts in our Ghanaian cohort. Our analysis found association of several HLA alleles with higher viral loads or lower CD4 counts. Also, we found association of several HLA alleles with lower viral loads or higher CD4 counts. In particular, *HLA-B*15:10* was associated with both higher viral loads and lower CD4 counts, which strongly suggests that this allele may be detrimental for HIV control. Our progress facilitates high-resolution HLA genotyping, contributing to our understanding of the HIV and host HLA interaction in Ghana, West Africa.

Current studies have indicated association of gut microbiome composition with varieties of disorders including infectious diseases. The microbiome composition is different among races and countries, possibly resulting in diversified interaction between host immune system and gut microbiome. However, data on gut/fecal microbiome have not been accumulated in West Africa. In this project, a technique for fecal microbiome analysis using NGS was introduced into Noguchi Memorial Institute for Medical Research (NMIMR). We examined fecal microbiome composition in healthy adults in Ghana, presenting the first substantial fecal microbiome data obtained by using high throughput metagenomic tools in Ghana. These data are valuable as a basis for determination of the association between fecal microbiome and progression of varieties of diseases in West Africa. These data and samples will become a part of the biobank program planned and initiated by the current SATREPS.

Further Achievements:

Ghanian researchers and students who learned technical and theoretical skills for molecular biology, microbiology, immunology and computational analysis contributed to the progress of this project. Two Ghanian researchers who obtained PhD though the current SATREPS project are continuously

working and contributing for the progress of this project in both Accra and Tokyo. It should be noted that under the unfortunate SARS-CoV-2 caused pandemic situation, they have provided their virology expertise on the planning and initiation of laboratory test system and played critical roles for COVID-19 diagnosis at the NMIMR after returning to Ghana. One Ghanaian researcher who obtained PhD is formally appointed as a junior researcher at National Institute of Infectious Diseases (NIID) in Japan and continuously working on the progress made for this project. Despite the COVID-19 pandemic, he has been back and forth between Tokyo and Accra multiple times for the continuation and advancement of our project. Because of such dedication and commitment made by our trained and talented Ghanian investigator(s), the project was able to maintain smooth and productive interactions with Ghanian researchers and investigators in both NMIMR and GHS under the difficult environment caused by the SARS-CoV-2 pandemic. It should be reemphasized that they did not just provide technical support for the STREPS project but also contributed the COVID-19 test and diagnosis together with the NMIMR dedicated investigators.

A recent infection testing algorithm (RITA) was introduced into estimation of the rate of early HIV diagnosis in Ghana. Early diagnosis with early treatment is important for the control of HIV epidemic, but our analysis showed low rate of early HIV diagnosis in Ghana, indicating the requirement of further evaluation of early HIV diagnosis rate.

Because of accurate HLA typing, we found new HLA allele candidates, which are now subjected to further analysis for determination. Furthermore, we determined multiple HIV genome polymorphisms associated with HLA genotypes. These data on viral and host polymorphisms are valuable as basic information for future development of novel drugs and vaccines.

Analyses of gut/fecal microbiome in individual regions are important for our understanding of pathogenesis in HIV infections. However, data on gut/fecal microbiome has not yet been accumulated in West Africa. We therefore examined fecal microbiome compositions in HIV-infected Ghanaian adults and compared the data with those in healthy HIV-negative Ghanaian adults. Analysis found that alpha diversity of fecal microbiome in HIV-infected individuals was significantly reduced compared to HIV-negative and associated with CD4 counts. HIV-infected individuals showed reduction in varieties of bacteria including *Faecalibacterium*, the most abundant in HIV-negative

controls, but enrichment of *Proteobacteria*. Further analysis revealed the characteristics of dysbiotic fecal microbiome in HIV infected adults in Ghana. This is the first study that analyzed the fecal microbiome in HIV-infected Ghanaian adults using high throughput metagenomic tools. Our results describing the feature of fecal microbiome in Ghana provide valuable data in West Africa, where data on enteric microbiome has not yet been systematically investigated and accumulated, and thus contribute to understanding of the interaction between HIV and enteric microbiota in a population specific manner.

Output 3: Domestic and international networks of infectious disease surveillance and related research are strengthened..
Activities: 3-1. To conduct quarterly knowledge sharing- and coordination meetings on surveillance, research findings, outcomes and evidence derived from the project activities. 3-2. To hold the "Scientific Conference" regularly to share and discuss information on progress and achievements of the international joint research Ghanaian and Japanese project implementation organizations (some of which are expected to be held as international workshops, with the participation of researchers and/or administrative officer(s) in charge of infectious disease control in nearby countries and representatives from African CDC). 3-3. Support surveillance in the application of knowledge acquired through basic research. 3-4. To conduct information sharing and discussion with relevant organizations on the findings, outcomes and achievements of the Project by participating in the meetings regarding infectious disease control, held by relevant organizations such as the Africa CDC as applicable. 3-5. To hold a dissemination conference for the deployment of the basic research-linked surveillance system to other regions and the addition of target pathogen(s) to the system, geared to the MOH, Regional Health Directorates of other regions and other organizations engaged in the research and/or control of infectious diseases.
Achievements: 3-1. ・ We had meetings with the Koforidua Hospital Team during project period

to share data on HIV/HLA/Microbiome.

- JICA long/short term experts and JICA coordinators provided their efforts to create mutually beneficial and trustable relationship with Ghanaian colleagues for advancing and sharing our knowledge in the area of HIV-HLA-Microbiome interactions. The established relationship will contribute to the sustainability of Ghana and Japan collaborative research on the HIV-HLA-Microbiome for further understanding of controlling HIV infection in Ghana and the rest of African countries.
- Through the STREPS project, we were able to establish the interactive platform with African CDC (see below in details).

3-2.

- Kick-Off Meeting and Scientific Symposium “Project for Surveillance and Laboratory Support for Emerging Pathogens”, Accra, Ghana, Sep. 27, 2016.
- SATREPS Scientific symposium “Human, Pathogen and Commensal Genomes for Future Surveillance”, Tokyo, Japan, Jan. 25-26, 2017.
- Symposium on Infectious Disease and Immunity on the occasion of the formal presentation ceremony of Dr. Hideyo Noguchi’s autopsy record from Ghana to Japan, Fukushima, Japan, June 9, 2018.
- SATREPS Scientific symposium on “Human, Pathogen and Commensal Genomes or Future Surveillance”, Accra, Ghana, Nov. 18, 2018.
- International Conference "Sustaining Global Health Gains through Partnerships in Biomedical Research" under Noguchi Memorial Institute for Medical Research 40 anniversary, Accra by hybrid conference protocol at Noguchi Memorial Institute for Medical Research, Ghana, Nov. 28-29, 2019.

3-3.

- Techniques for NGS, HLA typing, and microbiome analyses were introduced into NMIMR as described above. Analysis of diarrhea samples using these techniques found a group of bacteria that were not previously known to exist in Ghana, such as *Campylobacter* genus. Furthermore, we found that diarrhea attributed to virus infection (e.g., norovirus and rotavirus) rather than bacterial infection is unexpectedly predominant. Based on these important findings, examination of these bacteria and viruses was introduced into laboratory tests for determination of pathogens in diarrhea, contributing greatly to accurate pathogen diagnosis and control

of infectious diseases in Ghana. 3-4 ・ Through the SATREPS project, together with our NMIMR colleagues, we established the relationship with CDC by the constructive discussion with Dr. John N. Nkengasong Director, African CDC for the creation of SATREPS session during the annual African CDC meeting in order to present and share the different SATREPS projects and their progress. ・ International Conference on Re-Emerging Infectious Diseases (ICRED), SATREPS Session coordinated by JICA, AMED & Africa CDC, Addis Ababa, Ethiopia, March 18 – 20, 2020 postponed because of COVID-19 pandemic. 3-5. ・ Presentation by Hiroshi Kiyono at the 5th International Joint Symposium: The Promotion of Infectious Disease Research Cooperation between Africa and Japan Toward Science, Technology and Innovation (STI), AMED, Virtual, Nov. 5, 2020. ・ Presentation by Hiroshi Kiyono at the AMED International Joint Symposium 2021: Promotion of Infectious Disease Research Cooperation between Africa and Japan, Virtual, Nov. 1, 2021. ・ Establishment of fecal sampling system for microbiome analysis of healthy subjects and infectious disease patients in Ga West and Koforidua as model districts for the understanding of influence of gut microbiota on health and diseases of Ghanaian.
Further Achievements: Diarrhea surveillance system was reinforced by the progress of basic research and a package of surveillance guideline and training system was established in collaboration with GHS and NMIMR to be officially applied into infectious diseases surveillance in other areas in Ghana and neighbor countries in West Africa.

2. Achievements of the Project

2-1 Outputs and indicators

Output 1: A sustainable, basic research-linked surveillance for events of public health importance is established.
Indicators: 1-1. The standard sampling grade of approx. 10% in the sentinel surveillance

with necessary clinical information is maintained. 1-2. The practical operation of a prototype of sample banking system is commenced in the NMIMR. 1-3. The laboratory test results of causative pathogens including drug susceptibility information provided by the National Public Health Reference Laboratory (NPHRL) and/or the NPHRL have been fed back to the Ga West District Health Directorate within 10 days. 1-4. The guidelines for the introduction and operation of the basic research-linked surveillance system model are developed.
Achievements: 1-1. Activities on reinforcement of a surveillance system at the model area achieved satisfactory results about recording clinical information. Almost all the samples are sent with necessary clinical information for proper diagnoses. 1-2. A prototype of the sample storage system was constructed at NMIMR after the sampling and sample transfer system from community to laboratory was reviewed and changed into a functional system. The sample management system for SARS-CoV-2 will be expanded to cover all other samples in NMIMR. 1-3. The laboratory results were provided timely to clinicians and GHS officers on the field within 7 days from sample arrival. The diagnostic capacity at NPHRL was strengthened in regard to accuracy of laboratory diagnosis and rapidness of feedback. NMIMR will work for a cooperative institute of NPHRL when NPHRL needs technical supports in pathogen testing. 1-4. The products from these activities were packaged by GHS and NMIMR, and the package will be applied to the establishment of a surveillance system in other areas. The guideline of infectious disease surveillance was included in the package.
Further Achievements: A computer program for data analysis has been introduced to improve the surveillance system and disseminate the system extensively. Young scientists have been trained in Japan in the field of epidemiology and laboratory testing. They are expected to contribute to the maintenance and improvement of the surveillance system at the national level. The surveillance guideline package was used in two hospitals in the Greater Accra region and resulted in appropriate sampling. Under the chaos of COVID-19, samples are sent to Mie University and Mie National hospital, The laboratory investigation and training are ongoing with Ghanaian PhD students studying in Japan.

Output 2: The capacity of investigation and research for the identification of promoting, resistance and mucosal defense factors as well as the estimation of HIV endemicity status on the basis of genomic analyses of microbiome, HLA, enteric pathogens is strengthened in the NMIMR.
Achievements: NGS technique was introduced into NMIMR. Ghanian researchers who participated our SATREPS training courses or obtained PhD under our supervision in Japan learned skills for determination of pathogens, HLA genotyping, and microbiome analyses. In particular, accurate four-digit HLA typing system by a super high-resolution single-molecule sequence-based typing (SS-SBT) using NGS technique was introduced. Polymorphisms in HLA class I loci are known to have a great impact on disease progression in HIV infection. Prevailing HIV subtypes and HLA genotype distribution are different all over the world, and the HIV and host HLA interaction could be specific to individual disease areas. Data on the HIV and HLA interaction have been accumulated in HIV subtype B- and C-predominant populations but not fully obtained in West Africa where HIV subtype CRF02_AG is predominant. In this project, to obtain accurate HLA typing data for analysis of HLA association with disease progression in HIV infection in West African populations, HLA class I (*HLA-A*, *-B*, and *-C*) four-digit allele typing was performed in 324 treatment-naïve HIV infected individuals in Ghana by SS-SBT using next-generation sequencing. Comparison of the SS-SBT-based data with those obtained by a conventional sequencing-based typing (SBT) revealed incorrect assignment of several alleles by SBT (Figure 1). Indeed, HLA-A*23:17, HLA-B*07:06, HLA-C*07:18, and HLA-C*18:02 whose allele frequencies were 2.5%, 0.9%, 4.3%, and 3.7%, respectively, were not determined by SBT. Several HLA alleles were associated with clinical markers, viral load and $CD4^+$ T-cell count (Figure 2). Of note, the impact of *HLA-B*57:03* and *HLA-B*58:01*, known as protective alleles against HIV subtype B and C infection, on clinical markers was not observed in our cohort. This study for the first time presents SS-SBT-based four-digit typing data on *HLA-A, -B,* and *-C* alleles in Ghana, describing impact of HLA on viral load and CD4 count in HIV infection. Accumulation of these data would facilitate high-resolution HLA genotyping, contributing to our understanding of the HIV and host HLA

interaction in Ghana, West Africa.

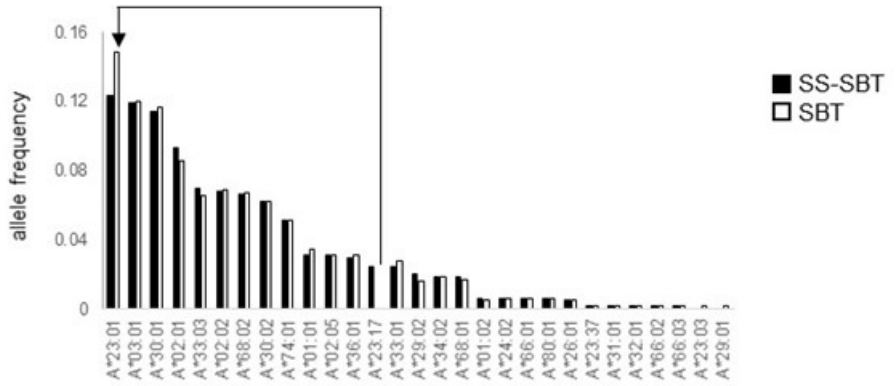

Figure 1. Comparison of HLA-A allele frequencies determined by SS-SBT and SBT in HIV infected Ghanaians.

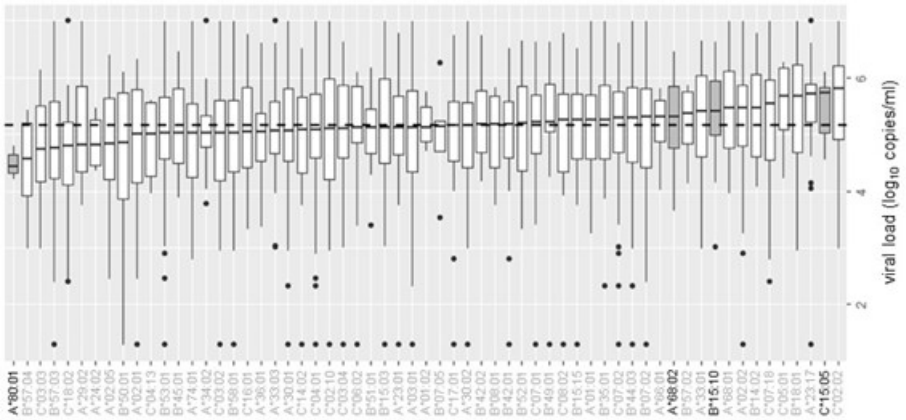

Figure 2. Correlation analysis between HLA alleles and viral loads in HIV infected Ghanaians.

Furthermore, the technical and theoretical skills for fecal microbiome analysis were introduced into NMIMR. Current studies have indicated association of gut microbiome composition with varieties of disorders including infectious diseases. The microbiome composition is different among races and countries, possibly resulting in diversified interaction between host immune and gut microbiome. Characterization of the baseline microbiota in healthy people is an essential step to understand this biological interaction in individual populations. However, data on gut/fecal microbiome has not been accumulated in West Africa. In this project, we examined fecal microbiome composition in healthy adults in Ghana. The 16S rRNA gene libraries were prepared using bacteria fractions derived from 55 Ghanaian adults and subjected to next generation sequencing. Fecal microbiome of Ghanaian adults was dominated

by *Firmicutes* (*Faecalibacterium*, *Subdoligranulum*, and *Ruminococcaceae UCG-014*), *Proteobacteria* (*Escherichia-Shigella* and *Klebsiella*), and *Bacteroidetes* (*Prevotella 9* and *Bacteroides*), consistent with previous observations in African cohorts. This is the first study that describes substantial fecal microbiome data obtained by using high throughput metagenomic tools in Ghana. These data would be valuable as a basis for determination of the association between fecal microbiome and progression of varieties of diseases in West African populations.

Further Achievements:

Because of accurate HLA typing, we found new HLA allele candidates, which are now subjected to further analysis for determination. Determination of new HLA alleles leads to further accurate HLA typing.

In HIV infections, cytotoxic T-lymphocyte (CTL) responses targeting HLA-restricted viral epitopes exert strong suppressive pressure on viral replication and frequently select for mutations resulting in viral escape from CTL recognition. Numerous data on these HLA-associated mutations in HIV subtypes B and C have been amassed with few reports described in other subtypes. In this project, we investigated HLA-associated mutations in HIV subtype CRF02_AG prevailing in Ghana, Western Africa. We determined viral *gag* sequences in 246 out of 324 HIV infected Ghanaians. Phylogeny analysis revealed that 200 (81.3%) were infected with HIV-1 CRF02_AG. Full *gag* and *vif* sequences were obtained from 199 and 138, respectively, out of the 200 individuals infected with CRF02_AG and subjected to determination of HLA-associated mutations. The analysis found HLA-associated HIV-1 CRF02_AG nonsynonymous polymorphisms at nineteen sites, thirteen in *gag* and six in *vif*, including those newly determined. Generation of this data is an important contribution to our understanding of HIV-1 CRF02_AG and host T cell interaction.

HIV infected individuals under antiretroviral therapy can control viremia but often develop non-AIDS diseases such as cardiovascular and metabolic disorders. Gut microbiome dysbiosis has been indicated to be associated with progression of these diseases. Analyses of gut/fecal microbiome in individual regions are important for our understanding of pathogenesis in HIV infections. However, data on gut/fecal microbiome has not yet been accumulated in West Africa. In this project, we examined fecal microbiome compositions in HIV infected adults in Ghana, where approximately two-thirds of infected adults are

females. In a cross-sectional case-control study, age- and gender-matched HIV infected adults (n = 55) and seronegative controls (n = 55) were enrolled (Figure 3). Alpha diversity of fecal microbiome in HIV+ was significantly reduced compared to HIV- and associated with CD4 counts (Figures 4 and 5). HIV+ showed reduction in varieties of bacteria including *Faecalibacterium*, the most abundant in seronegative controls, but enrichment of *Proteobacteria*. Ghanaian HIV+ exhibited enrichment of *Dorea* and *Blautia*; bacteria groups whose depletion has been reported in HIV infected individuals in several other cohorts. Furthermore, HIV+ in our cohort exhibited a depletion of *Prevotella*, a genus whose enrichment has recently been shown in men having sex with men (MSM) regardless of HIV status. The present study revealed the characteristics of dysbiotic fecal microbiome in HIV infected adults in Ghana, a representative of West African populations.

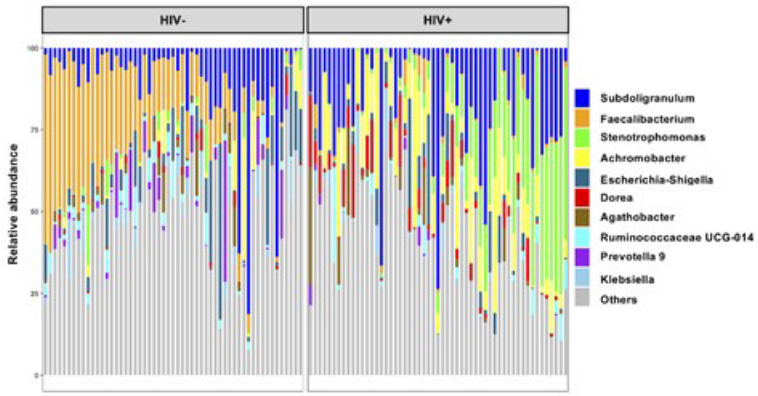

Figure 3. Top 10 abundant genera in fecal microbiome in uninfected (HIV-) and HIV infected (HIV+) Ghanaians.

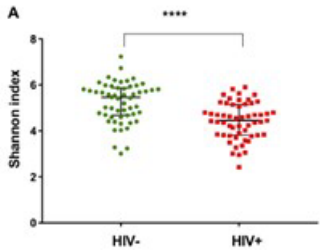

Figure 4. Comparison of Shannon diversities of fecal microbiome between HIV- and HIV+. HIV- showed significantly higher diversity than HIV+ ($p < 0.001$).

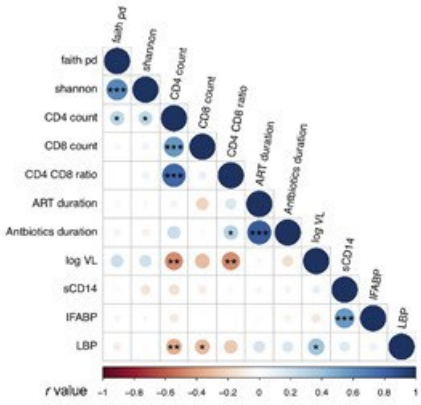

Figure 5. Analyses of correlation between fecal microbiome diversity and clinical markers.

Techniques for evaluation of early HIV diagnosis contributes to facilitating early HIV diagnosis and treatment for the control of HIV epidemic.

Output 3: Domestic and international networks of infectious disease surveillance and related research are strengthened.
Indicators:
Achievements: The base-line survey was conducted according to the WHO/AFRO guideline for IDSR. Based on the assessment, the surveillance system in Ga West was reinforced by performing training courses for health workers, arrangement of sample transfer methods from community to local and National laboratories and improving diagnostic test skills at these laboratories. The health center at the model area also launched a weekly epidemiological report on the major infectious diseases for health sectors. The system for accurate diagnosis with pathogen determination by cooperation between local laboratories and NPHRL in collaboration with NMIMR was established and strengthened.
Further Achievements: A package of surveillance guideline and training system was established in collaboration with GHS and NMIMR to be applied into infectious diseases surveillance in other areas in Ghana. Banking of samples obtained at surveillance was initiated. These samples are valuable as references for future surveillance and/or as controls for the development of novel drugs and vaccines.

2-2 Project Purpose and indicators

Project Purpose: A basic research-linked surveillance system model is established in Ghana
Indicators:
Achievements: Techniques for NGS, HLA typing, and microbiome analyses were introduced into NMIMR as described above. Analysis of diarrhea samples using these techniques found a group of bacteria that was not previously known to exist in Ghana, such as *Campylobacter* genus. Furthermore, we found that diarrhea attributed to virus infection (e.g., norovirus and rotavirus) rather than bacteria infection is unexpectedly predominant. Based on these important findings, analyses of these bacteria and viruses were introduced into laboratory tests for determination of pathogens in diarrhea, contributing greatly to accurate pathogen diagnosis and control of infectious diseases in Ghana.
Further Achievements: A package of surveillance guideline and training system was established in collaboration with GHS and NMIMR to be applied into infectious diseases surveillance in other areas in Ghana. Banking of samples obtained at surveillance was initiated. These samples are valuable as references for future surveillance and/or as controls for the development of novel drugs and vaccines.

3. History of PDM Modification

See Annex 3 as reference

4. Others

4-1 Results of Environmental and Social Considerations (if applicable)

Because of the global spread of the coronavirus disease 2019 (COVID-19), both Japanese and Ghanaian experts could not be dispatched as planned. However, we could overcome the unfortunate situation by the interactive platform between Ghanian and Japanese investigators as well as the efforts made by our Ghanian investigators and students for their courage and willingness to visit NMIMR and GHS to execute necessary experiments and data exchanges.

4-2 Results of Considerations on Gender/Peace Building/Poverty Reduction (if applicable)

One of Ghanian graduate students trained and obtained Ph.D in Japan through the current SATREPS project was officially hired by and appointed as a junior researcher at National Institute of Infectious Diseases in Tokyo. This definitively open new opportunities for other talented Ghanian students seeking continuous advancement of their scientific career and contributions for the sustainable bilateral scientific interactions between Ghana and Japan. Further, it will certainly contribute for advancing the diversified scientific talents and environments for Ghana and Japan corporative medical science.

III. Results of Joint Review

1. Results of Review based on DAC Evaluation Criteria

1.1 Relevance

The relevance of the Project was considered high based on the following information

- The diarrhea surveillance system was strengthened at the model area.
- NGS technique was introduced.
- Accurate HLA typing technique was introduced.
- Fecal microbiome analysis technique was introduced.

1.2 Coherence

The coherence of the project was considered high based on the following conditions.

- The country suffers from a high burden of infectious diseases, such as malaria, acute respiratory illness, HIV/AIDS, and diarrheal diseases. Ensuring the reduction of infections, especially among vulnerable groups are indicated in the national development plan “An Agenda for Jobs: Creating Prosperity and Equal Opportunity for All 2018-2021” In addition, “Health system strengthening” is identified as pillar under TICAD VI, and acceleration of action toward improving health surveillance, monitoring and evaluation are affirmed.
- This Project contributed for strengthening the surveillance and response system of infectious diseases which match to the national and regional development plan for health.

1.3 Effectiveness

The effectiveness of the project was considered high based on the achievement of the project purpose and the five outputs.

- A package of surveillance guideline and training system was established.
- The diarrhea diagnosis system was strengthened.
- Accurate HLA genotyping data in Ghanaians were obtained for the first time.
- Fecal microbiome data in healthy Ghanaians were obtained.
- Fecal microbiome data in HIV-1 infected Ghanaians were obtained.

1.4 Efficiency

The efficiency of the project was considered high based on analysis of inputs and outputs.

- The established package is useful for strengthening diarrhea surveillance system in Ghana and other countries in West Africa.
- Accurate diagnosis of diarrhea by viruses and non-cholera bacteria was facilitated.
- Association of HLA genotypes with viral load and disease progression in HIV infection was evaluated.
- HLA-associated HIV polymorphisms were determined.
- Dysbiosis of gut microbiome in HIV-1 infected Ghanaians was characterized.

1.5 Impact

Impact of the Project is expected to be positive as the results of the following analysis:

- The strengthened surveillance system could be applied into the surveillance of other infectious diseases including respiratory infections.
- The strengthened diagnosis techniques could be applied into other infectious diseases including respiratory infections.
- Data on pathogens, HLA, and microbiome could be the basis for the understanding of pathogenesis of infectious diseases and the development of vaccines and therapies.

1.6 Sustainability

The sustainability of the Project considered high based on the following information.

- Strong reliable relationship between researchers in Ghana and Japan was stablished.
- Many Ghanaian researchers learned skills for surveillance, diagnosis, NGS, HLA typing and microbiome analysis.

- Three Ghanaian researchers obtained PhD in Japan and are working for the control of infectious diseases with us in Ghana and/or in Japan. Two Ghanaian researchers are currently in the PhD course in Japan.
- The stored samples could be useful to further research for the control of infectious diseases.

2. Key Factors Affecting Implementation and Outcomes

- Strong reliable relationship between researchers and investigators in Ghana and Japan.
- Sustainable development of human resources.

3. Evaluation on the results of the Project Risk Management

- Establishment of strong reliable relationship between researchers and investigators in Ghana and Japan could be highly evaluated.
- Sustainable development of human resources could be highly evaluated.
- Activity to contribute to laboratory COVID-19 testing under COVID-19 pandemic could be highly evaluated.

4. Lessons Learnt

- Training and Education are important to develop human resources to play key roles in sustainable collaboration and research for the control of infectious diseases.
- The system for preparation of equipment and reagents needs to be improved.
- Stable electrical supply and network system should be strengthened.
- Daily management of purchasing the basic and clinical research related reagents and supplies needs to be simplified and improved.

IV. For the Achievement of Overall Goals after the Project Completion

1. Prospects to achieve Overall Goal

- In this project, the diarrhea surveillance system was strengthened at a model area, Ga West, and a package of surveillance guideline and training system was established in collaboration with GHS and NMIMR. This will be applied for strengthening diarrhea surveillance in other area, leading to strengthening diarrhea surveillance all over Ghana. Furthermore, this will also contribute to strengthening neighbor countries in West Africa.

- The package of surveillance guideline and training system described above can be applied into surveillance for other infectious diseases including respiratory infectious diseases, contributing to strengthening infectious disease surveillance in Ghana and possibly West Africa.
- In this project, laboratory techniques for pathogen diagnosis in diarrhea was strengthened at NPHRL and NMIMR with introduction of the technique for next generation sequencing (NGS) into NMIMR. Determination of pathogens in diarrhea will be continued and accumulation of data including those on microbiome will lead to establishment of more accurate and cost-effective diagnosis for diarrhea pathogens. Furthermore, these techniques can be applied for pathogen diagnosis in other infectious diseases, strengthening pathogen diagnosis techniques in infectious diseases and contributing to UHC.
- Training in this project resulted in improvement of accuracy of laboratory tests for HIV drug resistance. Furthermore, early HIV diagnosis rate was determined. These would be helpful to evaluation of activities for facilitating early diagnosis and early treatment toward HIV control.
- In this project, sample storage was initiated. Furthermore, data on HLA alleles and fecal microbiome compositions in Ghanaians were obtained by accurate HLA typing and analysis of fecal microbiome. These samples and data were important as a basis for determination of the impact of these factors on infectious diseases progression, understanding of the interaction of these factors with pathogens, and development of vaccines and therapies.
- In this project, reliable, respectable and stable relationship was established between the counterparts (NMIMR and GHS) and our Japanese team. Many Ghanaian talented researchers and students who learned technical and theoretical skills by training and/or PhD courses in Japan are continuously contributing their expertise to this project and/or collaborative research related to infectious diseases and their control. These would greatly contribute to future progress of our persistent collaboration toward establishment of Center for Excellence (COE) for infectious disease control in West Africa.

2. Plan of Operation and Implementation Structure of the Ghana side to achieve Overall Goal

- Utilizing the package of surveillance guideline and training system,

researchers in the counterparts are preparing introduction of the diarrhea surveillance system into other area in Ghana.

- Based on the intensive discussion with our respectful counterparts, we have decided to prepare a new application together for the next SATREPS project to apply the package developed by the current SATREPS project as described above for continuously strengthening the surveillance system for other infectious diseases, in particular respiratory infectious diseases.
- Our Ghanian colleagues are planning to strengthen laboratory techniques for pathogen diagnosis in other infectious diseases, in particular respiratory infectious diseases in continuous collaboration with us. We are fully committed for the interactive and productive collaboration with them.
- Drug-resistant HIV surveillance will be facilitated, which would be helpful to appropriate prescription for antiretroviral therapy (ART).
- To advance our knowledges and results generated by the current SATREPS for the control of infectious diseases in Ghana, both Ghanian and Japanese investigators have planned and organized a new application for the next SATREPS project to establish a biobank system to accumulate samples and data including those on HLA alleles and fecal microbiome compositions in Ghanaians, which could constitute a basis for the initiation of Ghana-originated new vaccine and therapy discovery program against infectious diseases.
- The current SATREPS project on the integrated surveillance and basic research established a unique interactive scientific platform for creating the Ghana-initiated new drug and vaccine discovery science. The trusted and respected interactive scientific relationships between Ghanaian and Japanese investigators are ready to move and advance the SATREPS project for the next stage for discovering new drugs and vaccines for the controlling infectious diseases in Africa by preparing and applying the next SATREPS project.

3. Recommendations for the Ghana side

- Introduction of the strengthened system of diarrhea surveillance into other area in Ghana is important and the package of surveillance guideline and training system would be very useful. Our Japanese team would be pleased to continuously work together with our Ghanian colleagues for accomplish our common goals.

- Application of the strengthened surveillance system built by the current SATREPS into surveillance of other infectious diseases, in particular respiratory infectious diseases, is logical and critical for the continuous improvement of infectious disease control in Ghana and neighboring countries. To this end, we have prepared a new application together for the next SATREPS project in order to continuously working together to achieve our common goal.
- Establishment of a biobank system would constitute a valuable scientific basis for starting Ghana-initiated new vaccine and therapy research in NMIMR. We have thus planed and prepared a new application together with NMIMR and GHS investigators for the next SATREPS project.

4. Monitoring Plan from the end of the Project to Ex-post Evaluation

- We do not have a system to monitor the progress of strengthening of diarrhea surveillance system in other areas in Ghana after completion of this project. However, because of our stable and reliable relationship with Ghanian colleagues, we will continue maintaining interactive communication with the counterparts for the continuous scientific interactions as well as training of next generation of investigators for achieving our common goal of the sustainable infectious disease control in Ghana and neighboring counties.
- Together with our respectable Ghanian counterparts, we have planned and prepared a new application for the next SATREPS project for strengthening of respiratory infectious disease surveillance and establishment of a biobank system for storage of samples and data which would constitute a valuable basis and asset for starting to build Ghana-initiated discovery of new generation of vaccines and therapies for the control of infectious diseases in Africa.

List of Dispatched Expert

Name of Experts		Date of Departure	Date of Arival	Affiliation	Title	Area of Expertise	
Adachi	Motoi	2016/08/06	2018/03/28	Mie University	Assoc.Prof.	surveillance	surveillance planning
Taniguchi	Kiyosu	2016/09/18	2016/09/25	National Mie Hospital	Director	surveillance	surveillance planning & protocol
Ishikawa	Koichi	2016/09/24	2016/09/29	National Inst.Infect.Dis.	Sen. Researcher	HIV	HIV research planning & kick of meeting
Matano	Tetsuro	2016/09/24	2016/09/29	Inst.Med.Sci, Tokyo Univ	Prof.	mucosal immunity	HIV research planning & kick off meeting
Kiyono	Hiroshi	2016/09/25	2016/09/29	Inst.Med.Sci, Tokyo Univ	Prof.	General Manager	surveillance planning & kick off meeting
Uematsu	Satoshi	2016/09/25	2016/09/29	Inst.Med.Sci, Tokyo Univ	Prof.	genome analysis	HIV research planning & kick of meeting
Ishikawa	Koichi	2016/11/21	2016/11/25	National Inst.Infect.Dis.	Sen. Researcher	HIV	surveillance planning & kick off meeting
Hori	Hiroki	2017/02/22	2017/03/02	Mie University	Prof.	surveillance	surveillance planning & kick off meeting
Ishikawa	Koichi	2017/02/25	2017/03/02	National Inst.Infect.Dis.	Sen. Researcher	HIV	HIV research protocol
Mizutani	Taketoshi	2017/02/25	2017/03/02	Institute of Microbial Chemistry	Researcher	microbiome	HIV research protocol
Mizutani	Taketoshi	2017/06/28	2019/09/27	Inst.Med.Sci, Tokyo Univ	Assis.Prof.	microbiome	HIV research protocol and planning
Ishikawa	Koichi	2017/08/22	2017/09/06	National Inst.Infect.Dis.	Sen. Researcher	HIV	meeting with new director of NMIMR
Kiyono	Hiroshi	2017/08/26	2017/08/30	Inst.Med.Sci, Tokyo Univ	Prof.	General Manager	meeting with new director of NMIMR
Ishikawa	Koichi	2017/11/13	2017/11/19	National Inst.Infect.Dis.	Sen. Researcher	HIV	Pre-JCC, Noguchi symposium

C/P Implementation List

Group Leader	Name	Institution	Position	research participating period			
				starts		ends	
				year	month	year	month
○	Prof. Abraham Kwabena Anang	Noguchi Memorial Institute for Medical Research	Director	2017	8	2021	7
	Prof. Kwadwo Ansah Koram	Noguchi Memorial Institute for Medical Research	Former Director/ PI on Surveillance	2016	8		
	Prof. William Kwabena Ampofo	Noguchi Memorial Institute for Medical Research	Head, Virology/PI on HIV	2016	8		
	Prof. Dorothy Kyerewah Yeboah Manu	Noguchi Memorial Institute for Medical Research	Former Head, Bacteriology/PI on Surveillance	2016	8		
	Prof. Kennedy Kwasi Addo	Noguchi Memorial Institute for Medical Research	Head, Bacteriology	2018	8	2021	7
	Dr. Evelyn Yayra Bonney	Noguchi Memorial Institute for Medical Research	Research Fellow, Virology	2016	8		
	Mr. Christian Bonsu	Noguchi Memorial Institute for Medical Research	Principal Technologist, Bacteriology	2017	3	2019	3
	Ms. Doris Aboagyewaa Boampong	Noguchi Memorial Institute for Medical Research	Research Assistant, Bacteriology	2017	3	2018	6
	Ms. Diana Asema Asandem	Noguchi Memorial Institute for Medical Research	Senior Research Assistant, Bacteriology	2018	6	2020	9
	Ms. Pheonah Badu	Noguchi Memorial Institute for Medical Research	Senior Research Assistant, Bacteriology	2018	1	2018	7
	Dr. Samuel Yaw Aboagye	Noguchi Memorial Institute for Medical Research	Chief Research Assistant, Bacteriology	2018	10	2019	3
	Mr. Abana Christopher Zaab-Yen	Noguchi Memorial Institute for Medical Research	Senior Research Assistant, Virology	2016	3		
	Dr. Sampson Badu Ofori	Noguchi Memorial Institute for Medical Research	Clinical Care Coordinator, Koforidua	2017	9		
	Mr. Dennis Kushitor	Noguchi Memorial Institute for Medical Research	Senior Research Assistant, Virology	2018	5		
	Mr. Theophilus Afum	Noguchi Memorial Institute for Medical Research	Senior Research Assistant, Bacteriology	2019	5		
	Mr. Abdul Basit Musah	Noguchi Memorial Institute for Medical Research	Research Assistant, Bacteriology	2021	1		
	Dr. Badu Sarkodie	Ghana Health Service	Director, Public Health	2016	8	2020	7
	Dr. Franklin Asiedu-Bekoe	Ghana Health Service	Head, Disease Surveillance Department	2016	8		

	Dr.Jhon Kofi Odoom	Ghana Health Service	Head, Disease Surveillance Department	2021	1		
	Mr. Kwame Owsu	Ghana Health Service	Data Manager, Disease Surveillance Department	2016	8		
	Dr. David Opare	National Health Reference Laboratory, Ghana Health Service	Head	2016	8	2020	7
	Dr. Gifty Boateng	National Health Reference Laboratory, Ghana Health Service	Duputy Head	2016	8		
	Dr. Doris Arhin	Ga West Municipality, Ghana Health Service	District Director	2016	12	2020	7
	Dr. Margaretta Gloria Chandi	Ga West Municipality, Ghana Health Service	District Director	2020	7		
	Mrs. Thelma Teley Aphour	Ga West Municipality, Ghana Health Service	Disease Control Officer	2016	12	2021	8

List of Equipment

Place	Equipment	Allocation	date of arrival	usage
Japan	High speed refrigerated centrifuge	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Accessory for NM-307	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Mini Personal Centrifuge, MultiSpin	NMIMR, Virology Department	20180810	HIV,microbiome, drug resistance
Japan	Mini Personal Centrifuge, OneSpin	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Pipette Calibration Station	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Fluorometer	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Heat Block	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Block	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Accessory for LCX-100	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Bioanalyzer	NMIMR, Virology Department	20180810	HIV, microbiome, drug resistance
Japan	Low Speed Bench-top Centrifuge	NMIMR, Bacteriology Department	20180810	microbiome, drug resistance, diarrhea study
Japan	Incubator	NMIMR, Bacteriology Department	20180810	microbiome, drug resistance, diarrhea study
Local	Photocopier	NMIMR Office	20160830	SATREPS project office
Local	Cabinet	NMIMR Office	20160920	SATREPS project office
Local	Laptop computer	GHS, Disease Surveillance Department	20161020	diarrhea surveillance
Local	Projector	GHS, Disease Surveillance Department	20161020	diarrhea surveillance
Local	Laptop computer	GHS, Disease Surveillance Department	20161116	diarrhea surveillance
Local	Laptop computer	NMIMR, Virology Department	20161213	HIV/AIDS data analysis
Local	Laptop computer	NMIMR, Bacteriology Department	20161213	diarrhea surveillance analysis
Local	Photocopier	GHS, Disease Surveillance Department	20161219	diarrhea surveillance
Local	Project Vehicle	NMIMR Office	20170110	SATREPS project activity
Local	Laptop computer	Eastern Regional Hospital, Koforidua	20170214	HIV/AIDS data analysis
Local	Refrigerator	Eastern Regional Hospital, Koforidua	20170809	HIV/AIDS sample storage
Local	Laptop computers	Ga West Municipal Hospital, Oduman Health Center, Mayera HC, Kotoku HC, St. John's Hospital, Ga West Management	20170904	diarrhea surveillance analysis
Local	Microscopes	Ga West Municipal Hospital, Oduman Health Center, Mayera HC, Kotoku HC, St. John's Hospital	20171004	diarrhea surveillance study
Local	Motorcycles	Oduman Health Center, Mayera HC, Kotoku HC	20171030	diarrhea surveillance, sample transportation
Local	Package of upgrade of 3130 genetic analyzer	NMIMR, Virology Department	20171114	HIV/AIDS DNA sequence
Local	Photocopier	Ga West District Office	20180205	diarrhea surveillance
Local	Refrigerator	National Health Reference Laboratory, Ghana Health Service	20190320	diarrhea surveillance sample storage
Local	Laptop computer	NMIMR, Bacteriology Department	20190521	diarrhea surveillance analysis
Local	Media Dispenser	National Health Reference Laboratory, Ghana Health Service	20190730	diarrhea surveillance study
Local	Microscan	NMIMR, Bacteriology Department	20191202	diarrhea surveillance, drug resistance
Local	RNA Extractor	NMIMR, Bacteriology Department	20200824	diarrhea surveillance, sample preparation
Local	Bio Banking System	NMIMR	20220124	Database

Technical transfer and knowledge/information sharing during SATREPS project

	Date	Participants	Programe	Location	Remarks
2017					
16-Jan	26-Jan	Dr.Badu Sarkodie Dr.Franklin Asiedu-Bekoe	Introduction of Japanese Surveillance system	Tokyo, Kawasaki, MOHW etc	
20-May	8-Jun	Mr.Christian Bonsu Mrs.Gifty Mawuli	NGS analysis for microbiome study	Tokyo	
13-Jun	15-Jun	GHS staff, Nurse, Technisian	ISDR training	Ga West	sample collection
17-Jul	18-Jul	GHS staff, Nurse, Technisian	ISDR training	Ga West	sample transportaion,Lecture and practice
2018					
29-Jan	17-Mar	Noguchi Staff	Microbiome study	Noguchi	by Dr.Aya Ishizuka,sample
27-Feb	20-Mar	Noguchi Staff	NGS microbiome analisis by PC software	Noguchi	by Mr.Prince Parbie (NIID)
12-Mar	27-Mar	Noguchi staff	HIV Drug resistance and HLA analysis	Noguchi	By Mildred A. Bosompen (NIID)
3-Jun	18-Jun	Prof.Kwabena Manta Bosompem (Anang) (Noguchi Director)	Public health policy, structure, system	Tokyo, Nagoya, Mie etc	Lecture of Prof.Anang was held in Quarantine station of Nagoya Airport.
12-Nov	23-Nov	Dr.Samuel Yaw Aboagye Dr.Isaac Darko Otchere Ms.Diana Asandem Mr.Christopher Zaab-Yen Abana Ms Stephen Nyarko Mr.Dennis Kushitor	16sRNA data analysis by Qiime2	Noguchi	Mr.Prince Parbie (NIID)
2019					
3-Jul	12-Jul	Ms. Christiana Kodom Achempem Ms. Thelma Tetteh (GHS staff)	Surveillance system and practice	Tokyo, Mie etc	
2021					

4-Feb	9-Mar	Noguchi Staff	diagnosis of viral diarrhea	Noguchi	Dr.Prince Parbie (NIID staff)
2-Jul	30-Jul	Noguchi Staff	HIV drug resistance, COVID diagnosis	Noguchi	Ms.Nana Afia (NIID)
17-Jul	9-Aug		HIV drug resistance, COVID diagnosisi	Noguchi	Dr.Prince Parbie (NIID Staff)
Sept.	Oct.	Virology Staff of Noguchi	HIV drug resistance practice and analysis	on line 10 times	Dr.Kikuchi, Dr.Ishikawa, Dr.Nishizawa Dr.Prince Parvie, Mr.Theodore (NIID) Africa Open Innovation Challenge powered by JICA
11-Nov	26-Dec	Virology Staff of Noguchi	HIV drug resistance analysis and database study COVID diagnosis	Noguchi	Dr.Prince Parbie (NIID staff)

Standard Operating Procedure (SOP) for enhanced diarrhea surveillance

- **General Description of enhanced diarrhea surveillance (EDS)**

This SOP is for enhanced diarrhea surveillance (EDS) at District level. The purpose of conducting EDS is to strengthen routine diarrhea surveillance in all health facilities in Ghana by timely investigating causative agent on regular sampling. Flow of sample and patient information along with the flow of feedback is summarized in Figure 1.

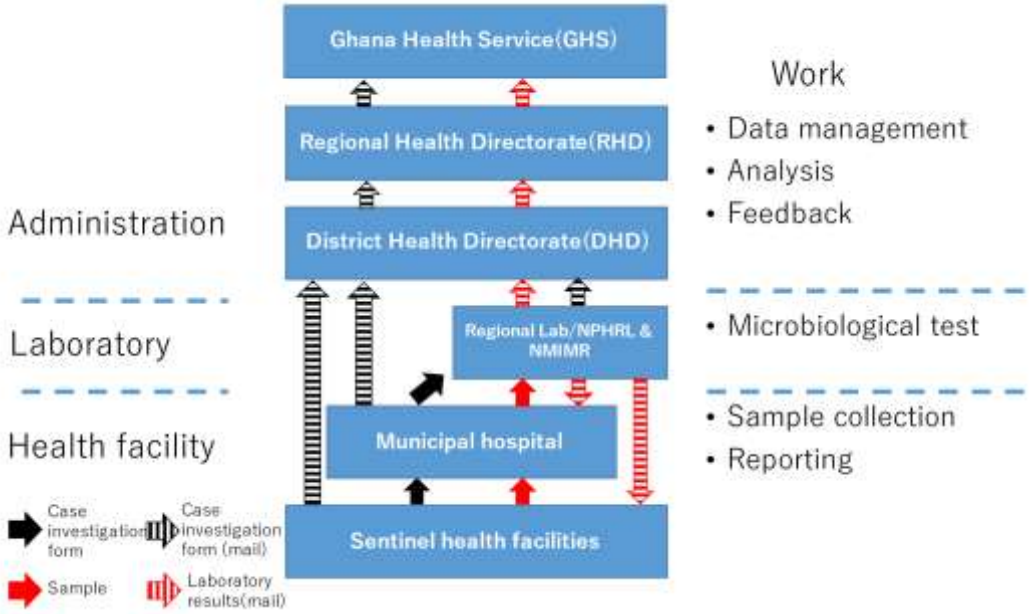

Fig 1. The overall of the EDS

At each sentinel site, every time a diarrhea patient visits their clinic, first the sample is tested for Cholera using rapid diagnosis test (RDT). If the test is positive, the case is treated as Cholera cases and investigation will be initiated. On the other hand, when RDT is negative, the patient is enrolled into EDS as acute diarrhea patient and both stool sample and patient information is collected. The health centers other than sentinel sites follows Ghana IDSR, i.e. the diarrhea patient is tested for cholera using RDT, if positive, active investigation, if negative, they are expected to report accumulated number of watery diarrhea cases weekly.

1. Select sentinel sites for enhanced diarrhea surveillance (EDS)

Sentinel sites for EDS should select nearest districts from 4 regional public health laboratories and National Public Health Reference Laboratory (NPHRL) or Noguchi Medical Institute for Medical Research (NMIMR). Based on population, prevalence of disease and other relevant information, the places and the number of sentinel sites(among tertiary hospital, municipal hospital, policlinic, Health center and private hospital) should be selected.

2. Train the health professionals and other eligible staff as well as community-based surveillance volunteers (CBSVs) in district for the introduction and operation of enhanced diarrhea surveillance (EDS)

Once you selected sentinel sites for EDS, you need to train the health professionals and other eligible staff as well as community-based surveillance volunteers (CBSVs) in district for the introduction and operation of EDS. In addition to this, you need to train data information officer to use EPI info to manage the data.

3. Case Definition for Diarrhea

Patients who meet the condition below:

Watery diarrhea (3 stools in 24 hours) with one of the following; ➢ Vomiting ➢ Fever with more than 37.5 C ➢ Abdominal pain

All the patients who met the criteria should undergo RDT for cholera and microscopy for parasite. If the test result is positive, follow the procedures for each disease. The patient will not be enrolled to EDS (Figure 2).

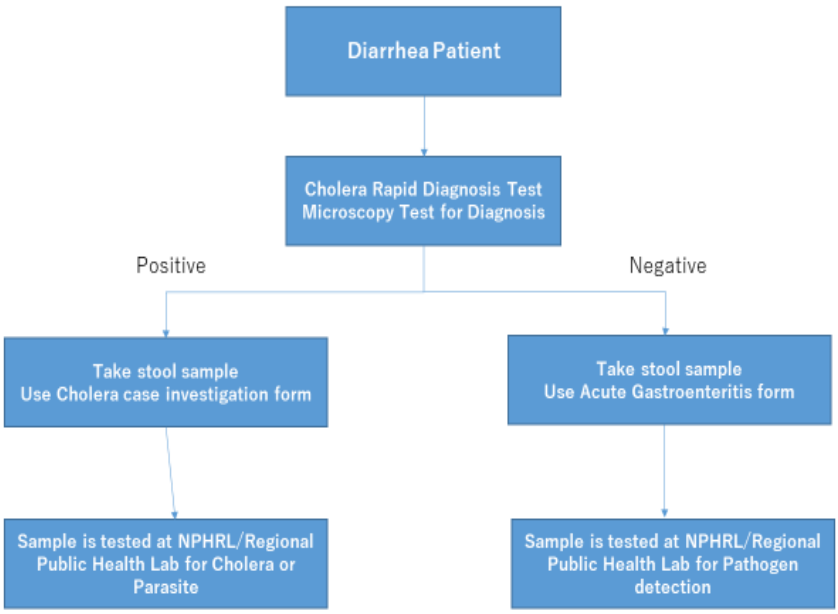

Figure 2. The flow of patient who visited medical facility with the symptom of diarrhea

4. Patient Information and stool specimen collection

For those who met the criteria for diarrhea and resulted negative for both cholera RDT and microscopy, is included in EDS. Once the patient is enrolled, patient information and stool sample should be collected.

1) Case-based information form

Once the diarrhea patient is ruled out for cholera and parasitic disease, the staff at the sentinel site will collect patient information using patient investigation form (Case-based information form: attachment 1).

- Fill all the information in the form except lab section.
- Attach the ID number sticker. Use the same ID number with stool sample taken from the patient.
- Enter the collected information into Epi info and download and save the information as a “line list” in excel sheet.

- Report patient information to District Health Directorate(DHD). During this step, send DHD the ONLY newly reported case from the previous report.

2) stool sample collection

Minimum requirement number for each site to collect stool sample is two per week. However, if you can collect more than two samples per week, it is OK to collect more than two.

- Priority is to collect stool sample because stool sample can provide additional information such as sensitivity analysis for antimicrobe, genotype, serogroup, etc.
- Attach the ID number sticker to the container. Use the same ID number with the patient information form of the same patient.
- Once you have collected the stool specimen, put it into the refrigerator until the motorbike or other transportations is ready to start.
- Try to collect samples from both groups (i.e. less than 5 years old, more than 5 years old). Ideally, the proportion of the number of diarrhea cases in each group and the number of the samples are nearly equal.

  Example: If the number of diarrhea case in adult for the week was 20 and children 10 (the ratio of 2:1), the sample taken from adult is 2 and from children 1 (the ratio is also 2:1) will be ideal.

5. Information form and sample transportation

1) All collected samples are sent to the nearest Regional Public Health Laboratory /NPHRL/NMIMR with case-based information form.

- Stool sample and case-based information form with the same ID will be transferred to the nearest Regional Public Health Laboratory /NPHRL/NMIMR.
- Put stool sample and case-based information form together into cooler box

(picture) with enough icepack so that stool sample is kept under 4℃ until it arrives at the nearest Regional Public Health Laboratory /NPHRL/NMIMR. Make sure the samples are well refrigerated during the transportation.

(example of cooler box)

- Do not separate stool sample and case-based reporting form even it has specific ID number.

2) Regional Public Health Laboratory /NPHRL/NMIMR will test for bacteria pathogen as well as virus pathogen.

6. Reporting (or Feedback)

One of the important purposes of this surveillance is to share the results with all the people involved in the surveillance. Thus, the reporting (or feedback) of the test results is important.

1) Data management

- At Sentinel Sites
  - All sentinel sites should enter the information using EPI Info to create the line list of your own facility.
  - If you gain additional information on patients, update as soon as possible and make sure let DHD information officer know where you updated.
  - Report information on just new case (a case reported in the week) once a week to DHD (it is better to have specific day of the week to report).

- At DHD
  - Merge all line listing data sent from each sentinel site at DHD (person in charge).
  - Line listing should be managed by EPI Info database according to the data management training course.
  - If there are missing data, data with obvious error, or any other questionable information on the line list, data manager need to contact sentinel site and check the information form.
  - Share line list database with DSD, Regional Health Directorate(RHD), Regional Lab/NPHRL (person in charge) regularly.

- At Regional Public Health Laboratory/NPHRL/NMIMR
  - Received samples should be tested at earliest convenience.
  - Regional Public Health Laboratory/NPHRL/NMIMR must immediately feedback the test result of cholera suspected case even if the result was negative.
  - Duration of feedback of laboratory result should be 3 working days after receipt of samples. If a pathogen of Public Heath importance is identified, immediately report to GHS, RHD and DHD.
  - Must fill out the all laboratory section of case-based information form.
  - Regional Public Health Lab/NPHRL/NMIMR merges both Virologic data and Bacteriological data and send to GHS, RHD and DHD once a week.

2) Data Analysis

- At DHD
  - Merge clinical data and laboratory data using Epi-info database.
  - Use “Time”, “Place”, “Person” as the basics of summarizing. Following is some of the example of the results expected from the data analysis.
    - ✧ summary of descriptive information of the patients
    - ✧ write epi-curve of watery diarrhea, bloody diarrhea and cholera to see the trend of diarrhea
    - ✧ calculate the incidence rates of diarrhea disease by sub-district
    - ✧ summarize laboratory data by detected agents and antibiotic

resistance information

- Results should be summarized as a weekly report.

3) Feedback and Communication (Figure 1)

- At sentinel sites
  - Once you received laboratory information and weekly summary from DHD, share with staff at the facility.
  - The results should be utilized for future treatment and public health response.
  - If the data is incorrect, notify DHD staff and discuss until the issue is solved.

- At DHD
  - Feedback laboratory results to each sentinel sites immediately.
  - Check the latest data and assess the situation. Report to upper level (RHD and DSD) if outbreak or any other signals for increasing risk of the disease is suspected.
  - Communicate with community and health facility using weekly report and/or emergency alert.

- At Regional Public Health Laboratory/NPHRL/NMIMR
  - NMIMR is responsible for technical support for EDS. NMIMR and Regional Public Health Laboratory/NPHRL should have Joint session on quality assurance regularly.

Remarks

Surveillance results must be used to improve the situation, detect outbreak early, and future prevention of the disease. High quality data is necessary to achieve this goal and the only way is all the participants to follow this SOP. This SOP will be revised regularly once we notice any obstacles.

研究開発の概要

本研究では、西アフリカの感染症克服への貢献を目指し、ガーナにおける腸管感染症を中心とする主要感染症のサーベイランスと診断検査体制の強化およびコレラ菌・HIV 等の病原体感染への腸管粘膜免疫の作用機序解明を目的とした研究を遂行した。

●ガーナにおける主要感染症のサーベイランス体制構築・強化

ガーナでは、顧みられない熱帯病のみならず、コレラを初めとする腸管感染症、エイズ等の感染症の流行が持続しており、近隣国でのエボラウイルス病のアウトブレイクの発生もあり、国際的な感染症対策の面からも体制の強化が求められている。一方では、途上国において大きなインパクトのある下痢症でさえ、コレラ以外ではどのような病原体が問題になっているかわかっておらず、特に感染症のサーベイランス体制の確立および診断・検査体制の強化に結びつく取組みへのニーズが大きい。本事業ではサーベイランス全体の枠組みの強化を目指して、下痢症・HIV 感染症等の主要感染症を対象とし、ガーナにおけるサーベイランス体制の構築・強化を目的として本事業を実施した。

まず、ガーナにおける下痢症・出血熱・HIV 感染症等の主要感染症に関する現行の感染症サーベイランス体制の問題点を把握するため、野口記念医学研究所（野口研）、ガーナ保健省［Ghana Health Service(GHS)］の公衆衛生局サーベイランス部門と協力して、現状のサーベイランス体制とトレーニングプログラムの評価を行った。その検討結果に基づき、下痢症サーベイランスモデル地域を設定し、その結果をふまえてガーナ全国におけるサーベイランス体制（National Surveillance System）を構築する方針とした。

過去にコレラの蔓延があったグレーターアクラ州の Ga West Municipality（以下ガ・ウエスト郡）を下痢症サーベイランスモデル地域に設定し、地域内の医療機関において定点サーベイランス体制を整備した。その過程で、WHO/IHR の Core capacity assessment と WHO/AFRO の Integrated Disease Surveillance and Response（IDSR）評価指針によってサーベイランス体制の評価を行った。この評価結果から、現場での症例報告体制、この原因でもあるトレーニングと指導体制の不足、起因病原体が同定できておらず、これには検体採取、輸送方法、そしてこの地域の病原体検査をカバーする National Public Health Reference Laboratory（以下 NPHRL）の検査技術に課題が指摘された（評価結果については英文学術論文 1 報投稿中）。

これらの評価結果をもとに、サーベイランス事業に参加する担当者（医療従事者、コミュニティ・ヘルス・ワーカー）に対するトレーニングコースを実施し、地区から高次検査施設（郡病院、NPHRL、野口研）への検体輸送手順の確立と NPHRL の検査機能の強化、検査結果の迅速なフィードバックの導入を行った。これらの活動と並行し、地域の医療機関への迅速な情報提供のため、ガ・ウエスト郡保健所で疫学週報の作成を支援した。これらは最終的に GHS、野口研と共同でガイドラインとトレーニング資料のパッケージとしてまとめ、今後のガーナにおけるサーベイランスに関する共同

提言としてまとめる事ができガーナ政府・保健省の感染症対策指針に反映された事は大きな成果である。

検査診断技術の向上に関して、寄生虫については定点医療機関への顕微鏡設置とトレーニングにより現地での検査体制を整備し、検体検査については、採取された検体を野口研と NPHRL とに搬送し、野口研でウイルス（例、ノロウイルス、ロタウイルス、エンテロウイルス、アデノウイルス）と細菌（例、ビブリオ菌、サルモネラ菌、赤痢菌、大腸菌）そして NPHRL にて細菌検査を行った。これまで、ガーナでは下痢症の原因はコレラかノン・コレラかの病原体診断に留まっていたが、今回のプロジェクトにより、ウイルス検査体制が確立し、多くの下痢症がノロウイルスもしくはロタウイルス等のウイルスによるものであることが判明し、現場にフィードバックされた。今後、抗菌剤の適正使用につなげて行くことが肝要である。また、細菌検査は Vibrio 属、Salmonella 属、Shigella 属、そして大腸菌などの腸管細菌科細菌を培養できるようなシステムで検索を行ったが、野口研と NPHRL の解析結果に大きな齟齬が見つかり、日本から細菌学専門家を派遣し、現地での技術的な評価とともに病原体同定を目的とした培養トレーニングコースを行った。これにより NPHRL における基礎的な課題が指摘され、技術指導が行われた。これと前後して今後の検体検査において基本的なことは NPHRL で行い、より詳細な解析を野口研で行うという「サーベイランスと研究の連携」戦略に基づいて、ウイルス検査技術が野口研から NPHRL に移転された。

一方では本プロジェクトのもう一つの柱である、サーベイランスと研究との連携の活動の中で、下痢症検体を野口研にて網羅的な細菌遺伝子検索を行ったところ、多くの検体から Campylobacter 属の遺伝子が発見され、ガーナにおける細菌性下痢症において、コレラに次ぐ重要な病原体であることが示唆された（英文学術論文 1 報発表）。

ガ・ウエスト郡におけるフィードバック機能強化と次の定点サーベイランス展開予定地域であったグレーターアクラ州の担当者を対象に、サーベイランスデータの効果的なフィードバックを目標に、コンピュータプログラムによるデータ解析手法とグラフ化のトレーニングを行った。ガ・ウエスト郡における活動が完成しつつあり、病原体検査の技術移転がおおむね完了していたが、COVID-19 パンデミックの発生により、ガ・ウエスト郡での成果をもとにしたグレーターアクラ州へ拡大する計画に影響が出た。しかし、NPHRL の研究者がパンデミック下でもグレーターアクラ州での定点サーベイランスを少しずつ続け、検体を保存してくれたため、日本に輸送して検査をする事が出来た。さらに、2021 年に日本人専門家がガーナに渡航し、ガ・ウエスト郡における成果を基に、この定点サーベイランスをガーナの他の地域に展開するためのパッケージをガーナ保健省 GHS と野口研とで作成し、その後の展開を共同提言とすることで合意した。

●ガーナにおける主要感染症の病原体ゲノム検査体制強化と腸管粘膜感染防御に関する研究

ガーナをはじめとする西アフリカの感染症克服への貢献を目指し、まず、ガーナにおける下痢症・出血熱・HIV 感染症等の主要感染症の診断体制強化を目的として、病原体遺伝子診断技術を導入し、病原体ゲノム情報を集積した。また、腸管粘膜免疫の病原体感染への作用機序解明を目的とし、腸管感染症の代表的病原体であるコレラ菌と HIV に焦点を合わせ、病原体ゲノム、宿主ゲノ

ムおよび腸内細菌叢ゲノムの解析を推進した。これらの多様性の相関解析を展開して、感染症病態や腸管粘膜免疫等との関連を検討した。

下痢症・出血熱・HIV 感染症等の感染症について、東京大学医科学研究所・国立感染症研究所の各々の専門家が、ガーナ野口記念医学研究所の各担当者と緊密なコミュニケーションをとり、次世代シークエンス技術を導入するとともに、病原体遺伝子診断技術導入を進めた。HIV については、ウイルスゲノム情報収集に基づく薬剤耐性検査体制構築を推進するとともに、血清学的検査に基づき早期診断率を解析して、半年以内の早期診断は 10%に満たないことを示した（英文学術論文 1 報発表、1 報投稿中）。

コレラ菌・HIV 感染における腸管粘膜免疫の作用機序解明を目的とした研究では、コレラを含む下痢症患者、HIV 感染者および対照健常人の検体収集対象地域を選定して（Ga West および Koforidua）、血液および便検体収集を進め、次世代シークエンス技術を用いたヒト HLA 遺伝子型解析および腸内細菌叢マイクロバイオーム解析を行った。

HIV 感染者 300 名あまりの血液検体を用いた解析では、まずウイルスゲノムの塩基配列解析を行い、subtype AG が主要 HIV タイプであることを明らかにした。HLA 遺伝子型については、汎用されている sequence-based typing（SBT）による遺伝子型同定を進めた。しかし、ガーナを含む西アフリカにおける HLA 遺伝子型情報がこれまで十分に蓄積されておらず、一部ある既存の情報についても、さらに詳細に検討する必要がある為、次世代シークエンス技術を用いたより正確な解析法である super high-resolution single-molecule sequence-based typing（SS-SBT）を行った。その結果、SBT による誤った分類がいくつか認められ、SS-SBT 導入による精度の高い HLA typing の有用性が確認された。この SS-SBT を用いることにより、ガーナにおける精度の高い HLA 遺伝子型分布データを得るとともに、新規アレルを見出し同定中である。さらに、HIV 感染病態（血漿ウイルス量および末梢血 CD4 陽性 T 細胞数）との関連解析データを得て、他の subtype でみられる相関関係との相違点を見出した。また、細胞傷害性 T 細胞（CTL）逃避変異の指標である HLA 関連 HIV 変異（polymorphism）同定も行った。これらのデータは、西アフリ地域の HIV と宿主免疫との相互作用に基づくウイルス変化・進化ならびに感染免疫病態の理解に重要である（英文学術論文 1 報発表、1 報投稿中、1 報準備中）。

腸内マイクロバイオームは近年、各種疾患との相関が知られつつあり、感染症の感染動態・病態との関連解明は重要課題である。本研究では、まず、野口研において、便検体を用いた次世代シークエンサー活用による腸内マイクロバイオーム解析技術を導入・確立し、ガーナ人健常者 300 名と HIV 感染者 100 名から便検体を収集し、マイクロバイオーム解析を行った。一部の被験者からは経時的に便検体を収集した為、計 600 検体あまりの便検体となり、ガーナでは初めての便検体バンクとして今後の SATREPS 関連研究にも汎用できるバイオバンク立ち上げ計画に繋がった。その結果、ガーナ人特有の腸内マイクロバイオームデータを得るとともに、ガーナ HIV 感染者の腸内マイクロバイオームデータを初めて報告した。この便検体バンクを駆使して継続的に HIV 感染症感染病態と腸内マイクロバイオームとの関連に関する知見を蓄積しているところである（英文学術論文 2 報発表、1 報準備中）。

また、モデル地区として指定した Ga West における下痢症便検体についても次世代シークエンサーを用いた病原体同定解析を行い、Campylobacter 属といったこれまでガーナに存在が知られていなかった細菌群を検出したことは前述のとおりである(英文学術論文 1 報発表)。この結果を含め、次世代シークエンサーを用いた腸内マイクロバイオーム解析技術の野口研への導入とそれを運用する人材育成が、上記のサーベイランス・病原体診断体制の強化につながったことは重要な成果である。

腸内マイクロバイオームの菌叢は国ごと、人種ごとに異なることも知られている。また後天的な環境に影響を受けることもあり、腸管免疫と疾病との相関関係を理解する上で、人種を跨いだ比較解析が有効である。上述の通り、上記の解析からガーナ人特有の腸内マイクロバイオームの情報が得られつつあるが、これと比較する目的から、50 例の日本人の健常者および 100 例あまりの日本人 HIV 感染者の腸内マイクロバイオームデータを取得した(英文学術論文 1 報発表)。さらに、日本人検体を用いて、A 型肝炎感染や COVID-19 等についても腸内マイクロバイオームデータ蓄積を進めている(英文学術論文 1 報発表、1 報投稿中)。これらの成果は、腸内マイクロバイオームと感染動態・病態あるいは腸管免疫との相互作用の理解に基づく感染症制圧に向けた基盤情報として重要である。

本プロジェクトでは、サーベイランス・病原体診断や腸内マイクロバイオーム解析技術の導入など、ガーナにおける研究技術導入および研究者育成に貢献してきた。ガーナ野口研から日本への留学生の育成にも力を入れ、博士課程修了・学位取得者 3 名を輩出した。うち 2 名はガーナに帰国し感染症研究・対策に貢献中で、残り 1 名は感染研職員として本プロジェクトに貢献中である。さらに博士課程在学中ガーナ人 2 名も本プロジェクトに参加し、今後の本プロジェクトの成果を基盤とした持続性、さらには発展性において活躍することが期待される。

Achievement (Published Papers)

1. Kashima, K., Yuki, Y., Mejima, M., Kurokawa, S., Suzuki, Y., Minakawa, S., Takeyama, N., Fukuyama, Y., Azegami, T., Tanimoto, T., Kuroda, M., Tamura, M., Gomi, Y. and **Kiyono, H**. 2016. Good manufacturing practices production of a purification-free oral cholera vaccine expressed in transgenic rice plants. Plant Cell Rep. 35: 667-679.
2. Saito, S., Ainai, A., Suzuki, T., Harada, N., Ami, Y., Yuki, Y., Takeyama, H., **Kiyono, H.**, Tsukada, H. and Hasegawa, H. 2016. The effect of mucoadhesive excipient on the nasal retention time of and the antibody responses induced by an intranasal influenza vaccine. Vaccine 34: 1201-1207.
3. Yuki, Y., Kurokawa, S., Kozuka-Hata, H., Tokuhara, D., Mejima, M., Kuroda, M., Oyama, M., Nishimaki-Mogami, T., Teshima R. and **Kiyono, H.** 2016. Differential analyses of major allergen proteins in wild-type rice and rice producing a fragment of anti-rotavirus antibody. Regul. Toxicol. Pharmacol. 76: 128-136.
4. Joo, S., Fukuyama, Y., Park, E-J., Yuki, Y., Kurashima, Y., Ziegler, S.F. and **Kiyono, H.** 2016. Critical role of TSLP-responsive mucosal DCs for the induction of nasal antigen-specific IgA response. Mucosal Immunol. 10:901-911.
5. Shimokawa, C., Kanaya, T., Hachisuka, M., Ishiwata, K., Hisaeda, H., Kurashima, Y., **Kiyono, H**., Kaisho, T. and Ohno, H. 2017. Mast cells are crucial for group 2 innate lymphoid cells in helminthic infection. Immunity 46:863-874.
6. Ogawa, T., Kashima, K., Yuki, Y., Mejima, M., Kurokawa, S., Kuroda, M., Okazawa, A., **Kiyono, H.** and Ohta, D. 2017. Seed metabolome analysis of a transgenic rice line expressing cholera toxin B-subunit. Sci. Rep. 7:5196.
7. Kishikawa, S., Sato, S., Kaneto, S. Uchino, S., Kohsaka, S., Nakamura, S. and **Kiyono, H.** 2017. Allograft inflammatory factor 1 (Aif1) is a regulator of transcytosis in M cells. Nat. Commun. 8:14509.
8. Tada, R., Muto, S., Iwata, T., Hidaka, A., **Kiyono, H**., Kunisawa, J. and Aramaki Y. 2017. Attachment of class B CpG ODN onto DOTAP/DC-chol liposome in nasal vaccine formulation augments antigen-specific immune responses in mice. BMC Biotechnol. 10:68.
9. Furuta, Y., Tsai, SH., Kinoshita, M., Fujimoto, K., Okumura, R., Umemoto, E., Kurashima, Y, **Kiyono, H.**, Kayama, H. and Takeda, K. 2017. E-NPP3 controls plasmacytoid dendritic cell numbers in the small intestine. PLoS One. 12:e0172509.
10. Jinnohara, T., Kanaya, T., Hase, K., Sakakibara, S., Kato, T., Tachibana, N., Sasaki, T., Hashimoto, Y., Sato, T., Watarai, H., Kunisawa, J., Shibata, N., Williams, I.,

Kiyono, H. and Ohno, H. 2017. IL-22 BP dictates characteristics of Peyer's patch follicle-associated epithelium for antigen uptake. J. Exp. Med. 214:1607-1618.

11. Takahashi, Y., Sato, S., Kurashima, Y., Lai, C-Y, Otsu, M., Hayashi, M., Yamaguchi, T. and **Kiyono, H**. 2017. Reciprocal inflammatory signaling between intestinal epithelial cells and adipocytes in the absence of immune cells. EbioMedicine 23:34-45.
12. Koyanagi, N., Imai, T., Shindo, K., Sato, A., Fujii, W., Ichinohe, T., Takemura, N., Kakuta, S., Uematsu, S., **Kiyono, H.**, Maruzuru, Y., Arii, J., Kato, A. and Kawaguchi, Y. 2017. Herpes simplex virus-1 evasion of $CD8^+$ T cell accumulation contributes to viral encephalitis. J Clin Invest. 127:3784-3795.
13. Nii-Trebi NI, Brandful JAM, Ibe S, Sugiura W, Barnor JS, Bampoh PO, Yamaoka S, Matano T, Yoshimura K, Ishikawa K, Ampofo WK. J Med Microbiol. 2017, 66(11):1663-1672.
14. Takahashi, Y., Sato, S., Kurashima, Y., Yamamoto, T., Kurokawa, S., Yuki, Y., Takemura, N., Uematsu, S., Lai, C.Y., Otsu, M., Matsuno, H., Osawa, H., Mizushima, T., Nishimura, J., Hayashi, M., Yamaguchi, T. and **Kiyono, H.** 2018. A refined culture system for human induced pluripotent stem cell-derived intestinal epithelial organoids. Stem Cell Reports. 10:314-328.
15. Azegami, T., Yuki, Y., Hayashi, K., Hishikawa, A., Sawada, SI, Ishige, K., Akiyoshi, K., **Kiyono, H.** and Itoh, H. 2018. Intranasal vaccination against angiotensin II type 1 receptor and pneumococcal surface protein A attenuates hypertension and pneumococcal infection in rodents. J. Hypertens. 36:387-394.
16. Suzuki, H., Nagatake, T., Nasu, A., Lan, H., Ikegami, K., Setou, M., Hamazaki, Y., **Kiyono, H.**, Yagi, K., Kondoh, M. and Kunisawa, J. 2018. Impaired airway mucociliary function reduces antigen-specific IgA immune response to immunization with a claudin-4-targeting nasal vaccine in mice. Sci. Rep. 8:2904.
17. Takemura, N., Kurashima, Y., Mori, Y., Okada, K., Ogino, T., Osawa, H., Matsuno, H., Aayam, L., Kaneto, S., Park, E.J., Sato, S., Matsunaga, K., Tamura, Y., Ouchi, Y., Kumagai, Y., Kobayashi, D., Suzuki, Y., Yoshioka, Y., Nishimura. J., Mori, M., Ishii, K.J., Rothenberg, ME., **Kiyono, H.**, Akira, S. and Uematsu, S. 2018. Eosinophil depletion suppresses radiation-induced small intestinal fibrosis. Sci Transl Med. 21:10(429).
18. Hirukawa, S., Sagara, H., Kaneto, S., Kondo, T., Kiga, K., Sanada, T., **Kiyono, H.** and Mimuro, H. 2018. Characterization of morphological conversion of Helicobacter pylori under anaerobic conditions. Microbiol Immunol. 62:221-228.
19. Niimi, K., Usami, K., Fujita, Y., Abe, M., Furukawa, M., Suyama, Y., Sakai, Y., Kamioka, M., Shibata, N., Park, E.J., Sato, S., **Kiyono, H.**, Yoneyama, H., Kitazawa,

H., Watanabe, K., Nochi, T., Aso, H. 2018. Development of immune and microbial environments is independently regulated in the mammary gland. Mucosal Immunol. 11:643-653.

20. Shibata, N., Kunisawa, J., Hosomi, K., Fujimoto, Y., Mizote, K., Kitayama, N., Shimoyama, A., Mimuro, H., Sato, S., Kishishita, N., Ishii, KJ., Fukase, K., **Kiyono, H.** 2018. Lymphoid tissue-resident *Alcaligenes* LPS induces IgA production without excessive inflammatory responses via weak TLR4 agonist activity. Mucosal Immunol. 11:693-702.
21. Tada, R., Suzuki, H., Takahashi, S., Negishi, Y., **Kiyono, H.**, Kunisawa, Y. and Aramaki, Y. 2018. Nasal vaccination with pneumococcal surface protein A in combination with cationic liposomes consisting of DOTAP and DC-chol confers antigen-mediated protective immunity against *Streptococcus pneumoniae* infections in mice. Int. Immunopharm. 61:385–393
22. Tada, R., Hidaka, A., **Kiyono, H.**, Kunisawa, J. and Aramaki, Y. 2018. Intranasal administration of cationic liposomes enhanced granulocyte–macrophage colony-stimulating factor expression and this expression is dispensable for mucosal adjuvant activity. BMC Research Notes 11:472.
23. Tada, R., Yamanaka, D., Ogasawara, M., Saito, M., Ohno, N., **Kiyono, H.**, Kunisawa, J. and Aramaki, Y. 2018. Polymeric caffeic acid is a safer mucosal adjuvant that augments antigen-specific mucosal and systemic immune responses in mice. Mol Pharm. 15:4226-4234.
24. Sato, S., Hisaie, K., Kurokawa, S., Suzuki, A., Sakon, N., Uchida, Y., Yuki, Y. and **Kiyono, H.** 2018. Human norovirus propagation in human induced pluripotent stem cell-derived intestinal epithelial cells. Cell. Mol. Gastro. Hepatol. 7: 686-688.e5.
25. Kato, H., Kanou, K., Arima, Y., Ando, F., Matsuoka, S., Yoshimura, K., Matano, T., Matsui, T., Sunagawa, T., and Oishi, K. 2018. The importance of accounting for testing and positivity in surveillance by time and place: an illustration from HIV surveillance in Japan. Epidemiol. Infect. 146:2072-2078.
26. Hashizume-Takizawa, T., Shibata, N., Kurashima, Y., **Kiyono, H**, Kurita-Ochiai, T, and Fujihashi, K. 2019. Distinct roles for Peyer's patch B cells for induction of antigen-specific IgA antibody responses in mice administered oral recombinant *Salmonella.* Int Immunol. 31:531-541.
27. Fujimoto, K., Kawaguchi, Y., Shimohigoshi, M., Gotoh, Y., Nakano, Y., Usui, Y., Hayashi, T., Kimura, Y., Uematsu, M., Yamamoto, T., Akeda, Y., Rhee, J.H., Yuki, Y., Ishii, K.J., Crowe, S.E., Ernst, P.B., **Kiyono, H.** and Uematsu, S. 2019. Antigen-

specific mucosal immunity regulates development of intestinal bacteria- mediated diseases. Gastroenterology.157:1530-1543.e4.

28. Joo, S., Suwanto, A., Sato, A., Nakahashi-Ouchida, R., Mori, H., Uchida, Y., Sato, S., Kurashima, Y., Yuki, Y., Fujihashi, K., Kawaguchi, Y. and **Kiyono, H.** 2019. A role for the CCR5-CCL5 interaction in the preferential migration of HSV-2-specific effector cells to the vaginal mucosa upon nasal immunization. Mucosal Immunol. 2:1391-1403.
29. Tada, R., Ohshima, A., Tanazawa, Y., Ohmi, A., Takahashi, S., **Kiyono, H**., Kunisawa, J., Aramaki, Y. and Negishi, Y. 2019. Essential role of host double-stranded DNA released from dying cells by cationic liposomes for mucosal adjuvanticity. Vaccines (Basel) 8:8.
30. Hosomi, K., **Kiyono, H**. and Kunisawa, J. 2019. Fatty acid metabolism in the host and commensal bacteria for the control of intestinal immune responses and diseases. Gut Microbes. 23:1-9.
31. Takahashi, N., Matsuoka, S., Minh, T.T.T., Ba, H.P., Naruse, T.K., Kimura, A., Shiino, T., Kawana-Tachikawa, A., Ishikawa, K., Matano, T., and Thi, L.A.N. 2019. Human leukocyte antigen-associated gag and nef polymorphisms in HIV-1 subtype A/E-infected individuals in Vietnam. Microbes Infect. 21:113-118.
32. Adusei-Poku, M.A., Matsuoka, S., Bonney, E.Y., Abana, C.Z., Duker, E.O., Nii-Trebi, N.I., Ofori, S.B., Mizutani, T., Ishizaka, A., Shiino, T., Kawana-Tachikawa, A., Ishikawa, K., Ampofo, W.K., and Matano, T. 2019. Human leukocyte antigen-associated HIV-1 CRF02_AG *gag* and *vif* polymorphisms in Ghana. Jpn. J. Infect. Dis. 72:374-380.
33. Matsuoka, S., Nagashima, M., Sadamasu, K., Mori, H., Kawahata, T., Zaitsu, S., Nakamura, A., de Souza, M.S., and Matano, T. 2019. Estimating HIV-1 incidence in Japan from the proportion of recent infections. Prev. Med. Rep. 16:100994.
34. Christopher Z Abana , Kwamena W C Sagoe , Evelyn Y Bonney , Edward K Maina , Ishmael D Aziati , Esinam Agbosu , Gifty Mawuli , Linda M Styer , Koichi Ishikawa , James A M Brandful , William K Ampofo. 2019. Drug resistance mutations and viral load in human immunodeficiency virus type 2 and dual HIV-1/HIV-2 infected patients in Ghana. Medicine, 98(6):e14313.
35. Takahashi, I., Hosomi, K., Nagatake, T., Tobou, H., Yamamoto, D., Hayashi, I., Kurashima, Y., Sato, S., Shibata, N., Goto, Y., Maruyama, F., Nakagawa, I., Kuwae, A., Abe, A., Kunisawa, J., and Kiyono, H. 2020. Persistent colonization of non-lymphoid tissue-resident macrophages by *Stenotrophomonas maltophilia*. Int. Immunol. 32:133-141.

36. Isobe, J., Maeda, S., Obata, Y., Iizuka, K., Nakamura, Y., Fujimura, Y., Kimizuka, T., Hattori, K., Kim, Y-G., Morita, T., Kimura, I., Offermanns, S., Adachi, T., Nakao, A., Kiyono, H., Takahashi, D. and Hase, K. 2020. Commensal-bacteria-derived butyrate promotes the t-cell-independent iga response in the colon. Int. Immunol. 32:243-258.
37. Yuki, Y., Kurokawa, S., Sato, S., Sasou, A., Matsumoto, N., Suzuki, A., Sakon, N., Goda, Y., Takeyama, N., Miyoshi, T., Marcotte, H., Tanaka, T., Hammarstrom, L. and Kiyono, H. 2020. A heterodimeric antibody fragment for passive immunotherapy against norovirus infection. J Infect Dis. 222:470-478.
38. Nakamura, Y., Mimuro, H., Kunisawa, J., Furusawa, Y., Takahashi, D., Fujimura,Y., Kaisho, T., Kiyono, H. and Hase, K. 2020. Microfold cell-dependent antigen transport alleviates infectious colitis by inducing antigen-specific cellular immunity. Mucosal Immunol. 13:679-690.
39. Wang, Y., Hosomi, K., Shimoyama, A., Yoshii, K., Yamaura, H., Nagatake, T., Nishino, T., Kiyono, H., Fukase, K. and Kunisawa, J. 2020. Adjuvant activity of synthetic lipid A of alcaligenes, a gut-associated lymphoid tissue-resident commensal bacterium, to augment antigen-specific IgG and Th17 responses in systemic vaccine. Vaccines (Basel). 8:395.
40. Yoshii, K., Hosomi, K., Shimoyama, A., Wang, Y., Yamaura, H., Nagatake, T., Suzuki, H., Lan, H., Kiyono, H., Fukase, K. and Kunisawa, J. 2020. Chemically synthesized alcaligenes lipid A shows a potent and safe nasal vaccine adjuvant activity for the induction of *Streptococcus pneumoniae*-specific IgA and Th17 mediated protective Immunity. Microorganisms. 8:1102.
41. Fujimoto, K., Kimura, Y., Shimohigoshi, M., Satoh, T., Sato, S., Tremmel. G., Uematsu, M., Kawaguchi, Y., Usui, Y., Nakano, Y., Hayashi, T., Kashima, K., Yuki, Y., Yamaguchi, K., Furukawa, Y., Kakuta, M., Akiyama, Y., Yamaguchi, R., Crowe, S.E., Ernst, P.B., Miyano, S., Kiyono, H., Imoto, S. and Uematsu, S. 2020. Metagenome data on intestinal phage-bacteria associations aids the development of phage therapy against pathobionts. Cell. Host Microbe. 28:380-389.e9.
42. Hosomi, K., Shibata, N., Shimoyama, A., Uto, T., Nagatake, T., Tojima, Y., Nishino, T., Takeyama, H., Fukase, K., Kiyono, H. and Kunisawa, J. 2020. Lymphoid tissue-resident alcaligenes establish an intracellular symbiotic environment by creating a unique energy shift in dendritic Cells. Front Microbiol. 11:561005.
43. Osier, F., Ting, J.P.Y., Fraser, J., Lambrecht B.N., Romano, M., Gazzinelli, R.T., Bortoluci, K.R., Zamboni, D.S., Akbar, A.N., Evans, J., Brown, D.E., Patel, K.D., Wu, Y., Perez, A.B., Pérez, O., Kamradt, T., Falk, C., Barda-Saad, M., Ariel, A., Santoni, A., Annunziato, F., Cassatella, M.A., Kiyono, H., Chereshnev, V., Dieye, A., Mbow,

M., Mbengue, B., Niang, M.D.S. and Suchard, M., 2020. The global response to the COVID-19 pandemic: how have immunology societies contributed? Nat. Rev. Immunol. 10:594-602.

44. Matsumura, S., Kurashima, Y., Murasaki, S., Morimoto, M., Arai, F., Saito, Y., Katayama, N., Kim, D., Inagaki, Y., Kudo, T., Ernst, P.B., Shimizu, T., and Kiyono, H. 2020. Stratified layer analysis reveals intrinsic leptin stimulates cryptal mesenchymal cells for controlling mucosal inflammation. Sci. Rep. 10: 18351.
45. Takasato, Y., Kurashima, Y., Kiuchi, M., Hirahara, K., Murasaki, S., Arai, F., Izawa, K., Kaitani, A., Shimada, K., Saito, Y., Toyoshima, S., Nakamura, M., Fujisawa, K., Okayama, Y., Kunisawa, J., Kubo, M., Takemura, N., Uematsu, S., Akira, S., Kitaura, J., Takahashi, T., Nakayama, T. and Kiyono, H. 2020. Orally desensitized mast cells form a regulatory network with Treg cells for the control of food allergy. Mucosal Immunol. 14:640-651.
46. Sasou, A., Yuki, Y., Honma, A., Sugiura, K., Kashima, K., Kozuka-Hata, K., Nojima, M., Oyama, M., Kurokawa, S., Maruyama, S., Kuroda, M., Tanoue, S., Takamatsu, N., Fujihashi, K., Goto, E. and Kiyono, H. 2021. Comparative whole-genome and proteomics analyses of the next seed bank and the original master seed bank of MucoRice-CTB 51A line, a rice-based oral cholera vaccine. BMC Genomics. 22: 59.
47. Sato, H., Ota, Y., Kido, Y., Matsumoto, T., Matsubara, Y., Matano, T., Hirata, Y., Kawana-Tachikawa, A., Yamaoka, Y., Yotsuyanagi, H., and Adachi, E. 2020. Gut-homing CD4+ T cells are associated with the activity of gastritis in HIV-infected adults. AIDS Res. Hum. Retroviruses 36:910-917.
48. Parbie, P.K., Mizutani, T., Ishizaka, A., Kawana-Tachikawa, A., Runtuwene, L.R., Seki, S., Abana, C.Z., Kushitor, D., Bonney, E.Y., Ofori, S.B., Uematsu, S., Imoto, S., Kimura,Y., Kiyono, H., Ishikawa, K., Ampofo, W.K. and Matano, T. 2020. Fecal microbiome composition in healthy adults in Ghana. Jpn. J. Infect. Dis. 74:42-47.
49. Shimoyama, A., Flaviana, D.L., Yamaura, H., Mizote, K., Palmigiano, A., , Pither, M.D., Speciale, I., Uto, T., Masui, S., Sturiale, L., Garozzo, D., Hosomi, K., Shibata, N., Kabayama, K., Fujimoto, Y., Silipo, A., Kunisawa, J., Kiyono, H., Molinaro, A., and Fukase, K. 2021. Lipopolysaccharide from gut-associated lymphoid tissue-resident alcaligenes faecalis: Complete structure determination and chemical synthesis of its lipid A. Angew. Chem. Int. Ed. Engl. 60:10023-10031
50. Tada, R., Ogasawara, M., Yamanaka, D., Sakurai, Y., Negishi, Y., Kiyono, H., Ohno, N., Kunisawa, J., Aramaki, Y., 2021. Enzymatically polymerised polyphenols prepared from various precursors potentiate antigen-specific immune responses in both mucosal and systemic compartments in mice. PLoS One. 16:e0246422.

51. Fujimoto, K., Kimura, Y., Allegretti, J.R., Yamamoto, M., Zhang, Y.Z., Katayama, K., Tremmel, G., Kawaguchi, Y., Shimohigoshi, M., Hayashi, T., Uematsu, M., Yamaguchi, K., Furukawa, Y., Akiyama, Y., Yamaguchi, R., Crowe, S.E., Ernst, P.B., Miyano, S., Kiyono, H., Imoto, S. and Uematsu, S., 2021. Functional restoration of bacteriomes and viromes by fecal microbiota transplantation. Gastroenterology. 160:2089-2102.e12.
52. Kurashima, Y., Kigoshi, T., Murasaki, S., Arai, F., Shimada, K., Seki, N., Kim, Y.G., Hase, K., Ohno, H., Kawano, K., Ashida, H., Suzuki, T., Morimoto, M., Saito, Y., Sasou, A., Goda, Y., Yuki, Y., Inagaki, Y., Iijima, H., Suda, W., Hattori, M. and Kiyono, H. 2021. Pancreatic Glycoprotein 2 is a first line of defense for mucosal protection in intestinal inflammation. Nat. Commun. 12:1067.
53. Lee, J., Mohsen, A., Banerjee, A., McCullough, L.D., Mizuguchi, K., Shimaoka, M., Kiyono, H. and Park, E.J. 2021. Distinct age-specific miregulome profiling of isolated small and large intestinal epithelial cells in mice. Int. J. Mol. Sci. 22:3544.
54. Yuki, Y., Uchida, Y., Sawada, S., Nakahashi-Ouchida, R., Sugiura, K., Mori, H., Yamanoue, T., Machita, T., Honma, A., Kurokawa, S., Mukerji, R., Briles, D.E., Akiyoshi, K. and Kiyono, H. 2021. Characterization and specification of a trivalent protein-based Pneumococcal vaccine formulation using an adjuvant-free nanogel nasal delivery system. Mol Pharm. 18:1582-1592.
55. Mizutani, T., Aboagye, S.Y., Ishizaka, A., Afum, T., Mensah, G.I., Asante-Poku, A., Asandem, D.A., Parbie, P.K., Abana, C.Z., Kushitor, D., Bonney, E.Y., Adachi, M., Hori, H., Ishikawa, K., Matano, T., Taniguchi, K., Opare, D., Arhin, D., Asiedu-Bekoe, F., Ampofo, W.K., Yeboah-Manu, D., Koram, K.A., Anang, A.K., and Kiyono, H. 2021. Gut microbiota signature of pathogen-dependent dysbiosis in viral gastroenteritis. Sci. Rep. 11:13945.
56. Wang, Y., Hosomi, K., Shimoyama, A., Yoshii, K., Nagatake, T., Fujimoto, Y., Kiyono, H., Fukase, K., and Kunisawa, J. 2021. Lipopolysaccharide derived from the lymphoid-resident commensal bacteria *alcaligenes faecalis* functions as an effective nasal adjuvant to augment IgA antibody and Th17 cell responses. Front. Immunol. 12:699349.
57. Parbie, P.K., Mizutani, T., Ishizaka, A., Kawana-Tachikawa, A., Runtuwene, L.R., Seki, S., Abana, C.Z., Kushitor, D., Bonney, E.Y., Ofori, S.B., Uematsu, S., Imoto, S., Kimura, Y., Kiyono, H., Ishikawa, K., Ampofo, W.K. and Matano, T. 2021. Dysbiotic fecal microbiome in HIV-1 infected individuals in Ghana. Front. Cell Infect. Microbiol. 11:646467.

58. Nakahashi-Ouchida, R, Uchida, Y., Yuki, Y., Katakai, Y., Yamanoue, T., Ogawa, H., Munesue, Y., Nakano, N., Hanari, K., Miyazaki, T., Saito, Y., Umemoto, S., Sawada, S.I., Mukerji, R., Briles, D.E., Yasutomi, Y., Akiyoshi, K., and Kiyono, H. 2021. A nanogel-based trivalent PspA nasal vaccine protects macaques from intratracheal challenge with pneumococci. Vaccine. 39:3353-3364.

59. Yuki, Y., Nojima, M., Hosono, O., Tanaka, H., Kimura, Y., Satoh, T., Imoto, S., Uematsu, S., Kurokawa, S., Kashima, K., Mejima, M., Nakahashi-Ouchida, R., Uchida, Y., Marui, T., Yoshikawa, N., Nagamura, F., Fujihashi, K. and Kiyono, H. 2021. Oral MucoRice-CTB vaccine for safety and microbiota-dependent immunogenicity in humans: a phase 1 randomized trial. Lancet Microbe. (Published: June 25, 2021DOI:https://doi.org/10.1016/S2666-5247(20)30196-8)

60. Anzurez, A., Naka, I., Miki, S., Hosoya-Nakayama, K., Isshiki, M., Watanabe, Y., Nakamura-Hoshi, M., Seki, S., Matsumura, T., Takano, T., Onodera, T., Adachi, Y., Moriyama, S., Terahara, K., Tachikawa, N., Yoshimura, Y., Sasaki, H., Horiuchi, H., Miyata, N., Miyazaki, K., Koga, M., Ikeuchi, K., Nagai, H., Saito, M., Adachi, E., Yotsuyanagi, H., Kutsuna, S., Kawashima, A., Miyazato, Y., Kinoshita, N., Kouno, C., Tanaka, K., Takahashi, Y., Suzuki, T., Matano, T., Ohashi, J., and Kawana-Tachikawa, A. 2021. Association of *Human Leukocyte Antigen DRB1*09:01* with severe COVID-19. HLA 98:37-42.

61. Otani, M., Shiino, T., Kondo, M., Hachiya, A., Nishizawa, M., Kikuchi, T., Matano, T., and Japanese Drug Resistance HIV-1 Surveillance Network. 2021. Phylodynamic analysis reveals changing transmission dynamics of HIV-1 CRF01_AE in Japan from heterosexuals to men who have sex with men. Int. J. Infect. Dis. 108:397-405.

62. Ishizaka, A., Koga, M., Mizutani, T., Parbie, P., Prawisuda, D., Yusa, N., Sedohara, A., Kikuchi, T., Ikeuchi, K., Adachi, E., Koibuchi, T., Furukawa, Y., Tojo, A., Imoto, S., Suzuki, Y., Tsutsumi,T., Kiyono, H., Matano, T. and Yotsuyanagi, H. 2021. Unique gut microbiome in HIV patients on ART suggests association with chronic inflammation. Microbiology Spectrum. 9:e0070821.

63. Ishizaka, A., Koga, M., Mizutani, T., Lim, L.A., Adachi, E., Ikeuchi, K., Ueda, R., Aoyagi, H., Tanaka, S., Kiyono, H., Matano, T., Aizaki, H., Yoshio, S., Mita, E., Muramatsu, M., Kanto, T., Tsutsumi, T., and Yotsuyanagi, H. 2021. Prolonged gut dysbiosis and fecal excretion of hepatitis A virus in patients infected with human immunodeficiency virus. Viruses 13:2101.

64. 足立基，谷口清州，堀浩樹，水谷壮利，石坂彩，石川晃一，俣野哲朗，清野宏，Opare D, Arhin D, Asiedu-Bekoe F, Ampofo WK, Yeboah-Manu D, Koram KA, Anang AK. ーナにおける国際的に懸念される公衆衛生上の緊急事態に向けたサーベイランス強化とコレラ菌・

HIV 等の腸管粘膜感染防御に関する研究：地球規模課題対応国際科学技術協力プログラム（SATREPS）「学術プロジェクトと行政サービス強化との連携」. in submission

2. その他の著作物（総説、書籍など）

《SATREPS の成果として得られたその他の出版物などについて、著者名、タイトル、掲載誌もしくは書籍（誌名、巻、号、発表年）などを発行日順に記載してください。》

1. Lee, J., Park, E.J. and Kiyono, H. 2016. MicroRNA-orchestrated pathophysiologic control in gut homeostasis and inflammation. BMB Rep. 49: 263-9.
2. Goto, Y., Uematsu, S. and Kiyono, H. 2016. Epithelial glycosylation in gut homeostasis and inflammation. Nat Immunol. 17:1244-1251.
3. Nelson, S., Kiyono, H. and Kurashima, Y. 2017. Epithelial extracellular ATP: an initiator of immunity to parasitic infections. Immunol. Cell Biol. 95:117-118 Feb2017
4. Park, E.J., Shimaka, M. and Kiyono, H. 2017. MicroRNA-mediated dynamic control of mucosal immunity. Int. Immunol. 29:157-163.
5. Kurashima, Y. and Kiyono, H. 2017. Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. Annu. Rev. Immunol. 35:119-147.
6. Nakahashi-Ouchida, R., Yuki, Y. and Kiyono, H. 2017. Development of a nanogel-based nasal vaccine as a novel antigen delivery system. Expert Rev. Vaccines. 16:1231-1240.
7. Shibata, N., Kunisawa, J. and Kiyono, H. 2017. Dietary and microbial metabolites in the regulation of host immunity. Front. Microbiol. 8:2171.
8. Kurashima, Y., Yamamoto, D., Nelson, S., Uematsu, S., Ernst, P.B., Nakayama, T. and Kiyono, H. 2017. Mucosal mesenchymal cells: secondary barrier and peripheral educator for the gut immune system. Front. Immunol. 8: 1787.
9. Azegami, T., Yuki, Y., Nakahashi-Ouchida, R., Itoh, H. and Kiyono, H. 2018. Nanogel-based nasal vaccines for infectious and lifestyle-related diseases. Mol Immunol. 98:19-24.
10. Nakahashi-Ouchida, R., Yuki, Y. and Kiyono, H. 2018. Cationic pullulan nanogel as a safe and effective nasal vaccine delivery system for respiratory infectious diseases. Hum Vaccine Immunother. 14:2189-2193. doi: 10.1080/ 21645515.2018. 1461298.
11. Inami, A., Kiyono, H., and Kurashima, Y. 2018. ATP as a pathophysiologic mediator of bacteria-host crosstalk in the gastrointestinal tract. Int J Mol Sci. 2018. 19. pii: E2371. doi: 10.3390/ijms19082371.

12. Kurashima, Y., Tokuhara, D., Kamioka, M., Inagaki, Y. and Kiyono, H. 2019. Intrinsic control of surface immune and epithelial homeostasis by tissue-resident gut stromal cells. Front Immunol. 19:1281. doi: 10.3389/fimmu.2019.01281.
13. Kiyono, H. and Pascual, D.W. 2020. Mucosal Vaccines, Innovation for Preventing Infectious Diseases, Second Edition. Elsevier. ISBN: 978-0-12-811924-2, pp. 1-915

Appendix II

31st, March 2021

Project Design Matrix (PDM)（Version 3）

Project Duration: 5 years and 7 months from the 7th of August 2016 to the 6th of March 2022

Project Title: Surveillance and Laboratory Support for Emerging Pathogens of Public Health Importance in Ghana

Target Area: the Republic of Ghana

Implementing Agencies:

【Ghana】the Noguchi Memorial Institute of Medical Research (NMIMR) (representative agency), and the Ghana Health Service (GHS)

【Japan】the Institute of Medical Science, University of Tokyo, the Mie University, the National Mie Hospital and the National Institute of Infectious Diseases

Target Groups:

【Direct Beneficiaries】Researchers, administrative officers and health and laboratory professionals of the Project Implementing Agencies

【Indirect Beneficiaries】Greater Accra Region (Ga West) and Eastern Region (Koforidua) residents

Narrative Summary	Objectively Verifiable Indicators	Means of Verification	Important Assumptions	Achievemen	Remarks
Overall Goal					
The basic research-linked surveillance system model is expanded to other regions and applies other infectious diseases of public health concern in Ghana.	1. The basic research-linked surveillance system model is introduced to at least one other region, or the Integrated Disease Surveillance and Response (IDSR), to which the elements of the model were merged, is being operated as a standard of the infectious disease serveillance system in Ghna, in 3 years following the termination of the Project. 2. More than one alternative non-diarrhoeal infectious disease is applied to the basic research-linked surveillance system model within 3 years following the termination of the Project.	(1) Documents regarding the infectious disease surveillance (2) Other related documents			Activities incidental to the project researches such as animal experiments, utilization and access to biological recourses, recombinant DNA experiments, material transfer, biosafety, etc. shall be conducted in conformity to related international, domestic and organizational conventions, regulations and standards.
Project Purpose					
A basic research-linked surveillance system model is established in Ghana.	1. By the end of the project period, an endorsement was given to the basic research-linked surveillance system by Ghanaian authorities concerned such as the GHS and/or the MOH, or elements of the model are merged into the Integrated Disease Surveillance and Response (IDSR). 2. By the end of the project period, enteric disease research updates from the model district are published in GHS epidemiological bulletins as appropriate.	(1) Experts' project reports (2) Minutes of the Joint Coordinating Committee (JCC) (3) GHS epidemiological bulletins (4) Documents or other comparable materials explaining the endorsement of the guidelines or the integration to the IDSR. (5) Other project documents	1. The importance of the control and research of infectious diseases in the health-related policies is maintained in Ghana. 2. Other initiatives to measure zoonotic virus infections are appropriately implemented in Ghana.		
Outputs					
1 **A sustainable, basic research-linked surveillance system for infectious agents of public health importance is established.**	1-1. For 6 months from September 2020 to February 2021, the standard sampling grade of approx. 10% in the sentinel surveillance with necessary clinical information is maintained. 1-2. By February 2021, the practical operation of a prototype of sample banking system is commenced in the NMIMR. 1-3. For 6 months from September 2020 to February 2021, the laboratory test results of causative pathogens including drug susceptibility information provided by the National Public Health Reference Laboratory (NPHRL) and/or the NPHRL have been fed back to the Ga West District Health Directorate within 10 days. 1-4. By February 2021, the guidelines for the introduction and operation of the basic research-linked surveillance system model is developed.	(1) Experts' project reports (2) Minutes of JCC (3) Other project documents			

2	**The capacity of investigation and research for the identification of promoting, resistance and mucosal defense factors as well as the estimation of HIV endemicity status on the basis of genomic analyses of microbiome, HLA, enteric pathogens is strengthened in the NMIMR.**	2-1. By February 2021, at least 2 novel findings regarding the HIV genetic characterization are obtained. 2-2. By February 2021, at least 2 novel findings regarding the promoting, resistance and/or intestinal mucosal defense factors are obtained. 2-3. By the end of the project period, at least 3 papers from the following subjects published by Ghanaian researchers as first author or comparable responsibility with first author, in peer reviewed journals: microbiome; HIV; causative viral or bacterial pathogens of diarrhoea including drug susceptibility; HLA; promoting, resistance, mucosal defense factors of infections; and/or HIV genetic characterization.	(1) Experts' project reports (2) Minutes of JCC (3) Handouts and minutes of the Scientific Meetings (4) Research papers published in scientific journals (5) Other project documents
3	**Domestic and international networks of infectious disease surveillance and related research are strengthened.**	3-1. Quarterly knowledge sharing meetings are held with the participation of the NMIMR, the NPHRL, the GHS and other eligible organizations (as needed). 3-2. By 6 months before the end of the project period, at least one (1) international conference, workshop, etc. which are organized by the Project, is held.	(1) Experts' project reports (2) Minutes of JCC (3) Handouts and minutes of the Scientific Conferences (4) Other project documents

Activities	Inputs		Important Assumptions
1 **A sustainable, basic research-linked surveillance system for infectious agents of public health importance is established.**	**Japan**	**Ghana**	1. Ghanaian government accords sufficient budget and personnel to GHS and personnel to NMIMR. 2. Trained counterpart personnel turnover impact is limited. 3. Project implementation cooperation is obtained from health facilities and related stakeholder organizations.
1-1. Establishment of the sentinel surveillance system for diarrhoeal diseases	<u>Experts</u> (1) Chief Advisor (Short-term expert) (2) Surveillance (Long/short-term expert) (3) Microbiome Analysis (Long/short-term expert) (4) Project Coordinator (Long-term expert) (5) Experts for immunology and HIV (Short-term experts) <u>Training in Japan</u> (1) Surveillance (2) Molecular Biology <u>Equipment and materials</u> (1) Necessary equipment and reagents for research to be defined by research protocols (2) Necessary equipment and/or devices for collection and transport of specimens through early vigilance and rapid response mechanism *Local costs* Running expenses necessary for implementation of the project activities other than those that borne by the Ghanaian side	<u>Counterparts</u> (1) Project Director (2) Project Manager (3) Researchers in Bacteriology, Virology, Immunology, and Communicable disease surveillance (4) GHS surveillance staff (5) Research Assistants <u>Land, Facilities, equipment and materials</u> (1) Office space at NMIMR and GHS Surveillance (2) NMIMR laboratory facility access/use (3) Clinical specimens <u>Local costs</u> Running expenses necessary for implementation of the project activities such as personnel costs of permanent staff and utilities	
1-1-1. To conduct a baseline survey with developed tools in a selected model site.			
1-1-2. To assign 5 medical facilities (target facilities of the Project) as the sentinel sites of the surveillance of diarrhoeal diseases in the model site on the basis of the baseline survey.			
1-1-3. To provide the medical professionals and other eligible staff with trainings for the introduction and operation of the diarrhoea sentinel surveillance.			
1-1-4. To conduct training on surveillance geared to staff of local medical facilities and community-based surveillance volunteers (CBSVs) for the improvement of infectious disease surveillance in the entire model site, based on the results of the baseline survey.			
1-1-5. To develop Standard Operating Procedures (SOPs) for sample collection, data management and the feedback of test results including drug susceptibility of pathogens.			
1-1-6. To support the operational management of surveillance including sample collection and transport at communities and health facilities, monitoring & evaluation, and feedback, for the early detection of IDRS priority diseases.			
1-1-7. To transfer the detection techniques of major causative agents of viral diarrhoea such as rotavirus, norovirus, etc., as well as important bacteria (as needed basis) from the NMIMR to the NPHRL.			

1-2.	**Development of a prototype of sample banking system linked with the infectious disease surveillance**	
1-2-1.	To construct an information database of clinical samples collected through the passive and active surveillances (the diarrhoea surveillance and HIV research, respectively), consist of patient- and pathogen chraracgteristics including drug susceptibility in the NMIMR.	
1-2-2.	To arrange storage equipment for the preservation of clinical samples such as blood, feces, etc. as well as isolated and/or extracted DNA, pathogens, etc. in the NMIMR.	
1-2-3.	To develop regulations for the operation of a prototype of sample banking system, which stipulates not only the supply/transportation of clinical samples to the NMIMR, preservation, access and withdrawal, but also the maintenance management of the equipment and the information database.	
1-3.	**Functional verification of the basic research-linked surveillance system**	
1-3-1.	To review the basic research-linked surveillance system from the viewpoints of sustainability and applicability to other regions, followed by the modification of the system operation including the SOPs.	
1-3-2.	To verify the operability and effectiveness of the system on the basis of the IDSR performance indicators as well as the implementation of the simulation exercises (if needed), followed by the modification of it as needed basis.	
1-3-3.	To develop guidelines for the introduction to other regions and operation of the model by packaging the necessary procedures, training materials and its lecturers, operating expenses, timeframe, ledger sheets, etc.	
1-3-4.	To commence concrete discussions with Ghanaian authorities concerned such as the GHS for the endorsemenrt of the model or the mergence of the elements of it into the current IDSR.	
2	**The capacity of investigation and research for the identification of promoting, resistance and mucosal defense factors as well as the estimation of HIV endemicity status on the basis of genomic analyses of microbiome, HLA, enteric pathogens is strengthened in the NMIMR.**	
2-1.	To develop research protocols on HIV and diarrhoeal diseases in collaboration between Ghanaian and Japanese researchers.	
2-2.	To provide Ghanaian researchers with the trainings (theory and practice) of the analyses of pathogen genome in diarrhoea conditions, HLA-associated polymorphisms in Ghanaian HIV carriers, next-generation sequencing of microbiome and bioinformatics for the analyses on the interrelationship of the genomic diversity trilaterally (i.e., microbiome, pathogens and host) .	
2-3.	To perform the characterization of HIV infection genetically by performing the multiple analyses of HLA-associated polymorphisms and microbiome in Ghanaian HIV carriers with currently available data such as CD4+ T-cell conts and viral load as basic information for future development of novel drugs and vaccines.	

Pre-Conditions

2-4.	To identify promoting, resistance, mucosal defense factors of infections by analyzing the interrelationship of genomic diversity amongst microbiome, pathogens and host HLA, for future risk assessment of infections for severity, the estimation of the response to medications and so on.			The Ghanaian stakeholders engaged in the infectious disease control do not oppose to the commencement of the Project. ↓
3	**Domestic and international networks of infectious disease surveillance and related research are strengthened.**			**Issues and Countermeasures**
3-1.	To conduct quarterly knowledge sharing- and coordination meetings on surveillance, research findings, outcomes and evidences derived from the project activities.			
3-2.	To hold the "*Scientific Conference* " regularly to share and discuss information on progress and achievements of the international joint research Ghanaian and Japanese project implementation organizations (some of which are expected to be held as international workshops, with the participation of researchers and/or administrative officer(s) in charge of infectious disease control in nearby countries).			
3-3.	To conduct information sharing and discussion with relevant organizations on the findings, outcomes and achievements of the Project by participating in the meetings regarding infectious disease control, held by relevant organizations such as the Africa CDC as applicable.			
3-4.	To hold a dissemination conference for the deployment of the basic research-linked surveillance system to other regions and the addition of target pathogen(s) to the system, geared to the MOH, Regional Health Directorates of other regions and other organizations engaged in the research and/or control of infectious diseases.			

*: The basic research-linked surveillance system is a system in which laboratory diagnosis such as gene detection is carried out on clinical samples collected in sentinel sites (medical facilities), and the trend of infectious diseases and susceptibility information to antibiotics obtained by the mass analysis are fed back to local health administrative institutions and medical facilities, and then utilized for infection prevention measures and daily medical care in the region; simultaneously, the obtained clinical samples are registered and managed in the sample banking system accompanied with clinical information, and it can be utilized for basic research, which contributes to infection prevention, treatment and countermeasures in future.