SATREPS “The Project for the control of tuberculosis and glanders”

Project progress and future activities
- Research on glanders at Hokkaido University -

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Laboratory of Comparative Pathology

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Hokkaido University (HU), Japan

The 4th JCC. February 5’ 2025. Ulaanbaatar

Activities that FVM, HU should be responsible for

Dry-LAMP method

- **PDM 1.3.** Development of a LAMP-based Rapid diagnostic Method (Test Kit) for *B. mallei* infection
- **PDM 1.5.** To establish production systems for the genetic diagnostic kits described above by introducing Ink-jet printers into NCCD

Immunochromatography (ICT)

- **PDM 1.4.** Development of an immunochromatography-based Rapid Diagnostic Method (Test kit) for *B. mallei* infection

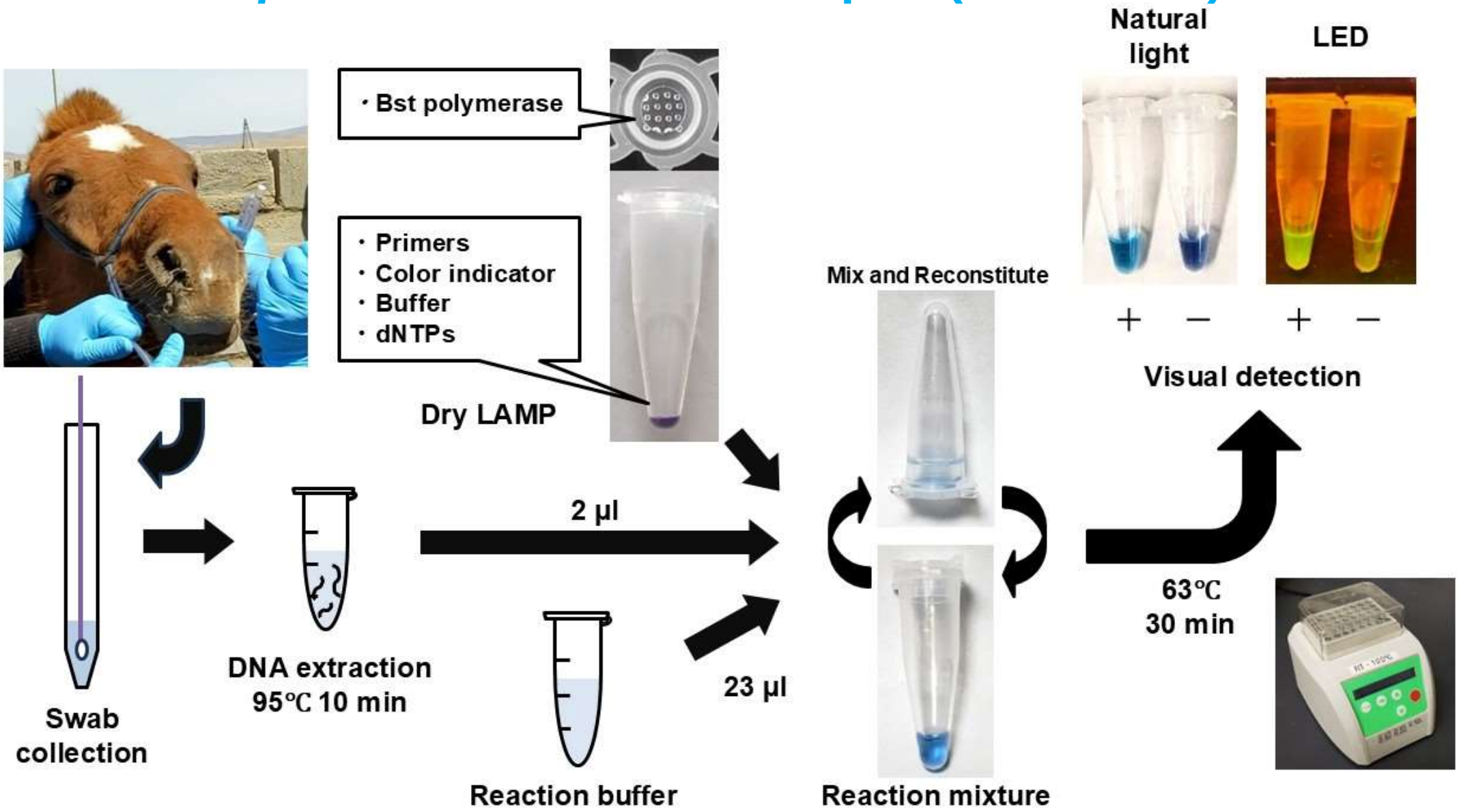
Activities that FVM, HU should be responsible for

Epidemiology

PDM 3.3. Molecular-epidemiological and seroepidemiological evaluation of the epidemics of *B. mallei* infection in horses

3.3.4. To assess the transmission and distribution of *B. mallei* in horses by performing the comprehensive gene screening using a next-generation sequencer on the isolated strains, which are obtained by culturing specimens of lesioned part of the infected horses.

PDM 1.3 A new dry-LAMP method for the specific detection of *B. mallei* and *B. psedomallei* was developed (2020-2023)

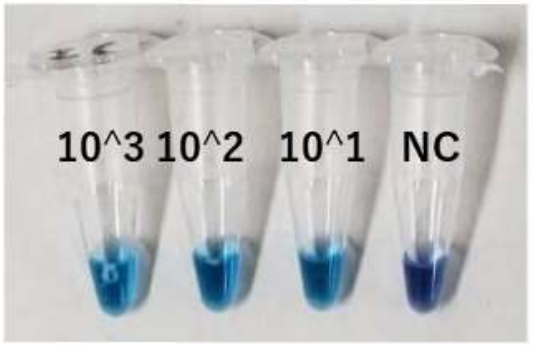


PDM 1.3 A new dry-LAMP method for the specific detection of *B. mallei* and *B. psedomallei* was developed (2020-2023)

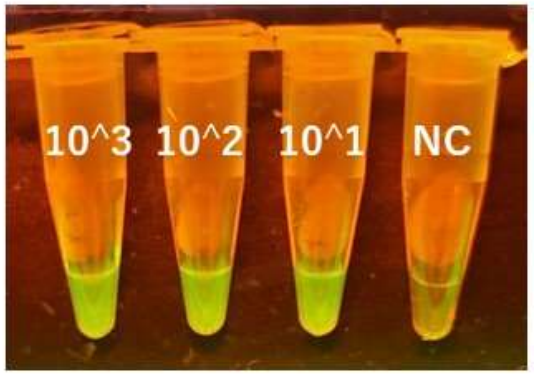
A

	Genomic copies per reaction		Time of positivity
B. mallei	1000	(n=2)	13.7±0.5
	100	(n=8)	15.4±0.5
	10	(n=8)	$16.9 \pm 1.3^{4/8}$
B. pseudomallei	100	(n=16)	$16.9 \pm 0.9^{11/16}$
B. thailandensis	1.36×10^5	(n=2)	NA
B. vietnamiensis	1.32×10^5	(n=2)	NA
B. cepacia	1.06×10^5	(n=2)	NA
NC	0	(n=4)	NA

B



natural right



LED

PDM 1.3 A new dry-LAMP method for the specific detection of *B. mallei* and *B. psedomallei* was developed (2020-2023)

		fliP-IS407A TaqMan PCR		
		Positive	Negative	Total
Bm/Bp dry LAMP	Positive	7	1	8
	Negative	1	10	11
	Total	8	11	19

Detection of *B. mallei* DNA in clinical samples (nasal and skin swabs from 19 *B. mallei*-infected horses)

- ✓ This is the first LAMP method capable of detecting *B. mallei* DNA in clinical specimens.
- ✓ It has diagnostic performance comparable to TaqMan PCR, which is recommended by WOAH.

Nakase *et al. BMC Microbiology* (2025) 25:36
https://doi.org/10.1186/s12866-024-03737-z

BMC Microbiology

RESEARCH Open Access

A novel ready-to-use loop-mediated isothermal amplification (LAMP) method for detection of *Burkholderia mallei* and *B. pseudomallei*

Mitsuru Nakase[1], Jeewan Thapa[2], Vanaabaatar Batbaatar[3], Ochirbat Khurtsbaatar[3], Batchuluun Enkhtuul[3], Jugderkhorloo Unenbat[3], Baasansuren Lkham[3], Sachiho Fujita[1], Ai Koshikawa[1], Apichai Tuanyok[4], Vannarat Saechan[5], Hideaki Higashi[6], Kyoko Hayashida[7], Yasuhiko Suzuki[2,8], Chie Nakajima[2,8] and Takashi Kimura[1*]

PDM 1.5 Training on making dry LAMP kits using the ink-jet printer installed at NRTL, NCCD (2023-2024)

September 14, 2023

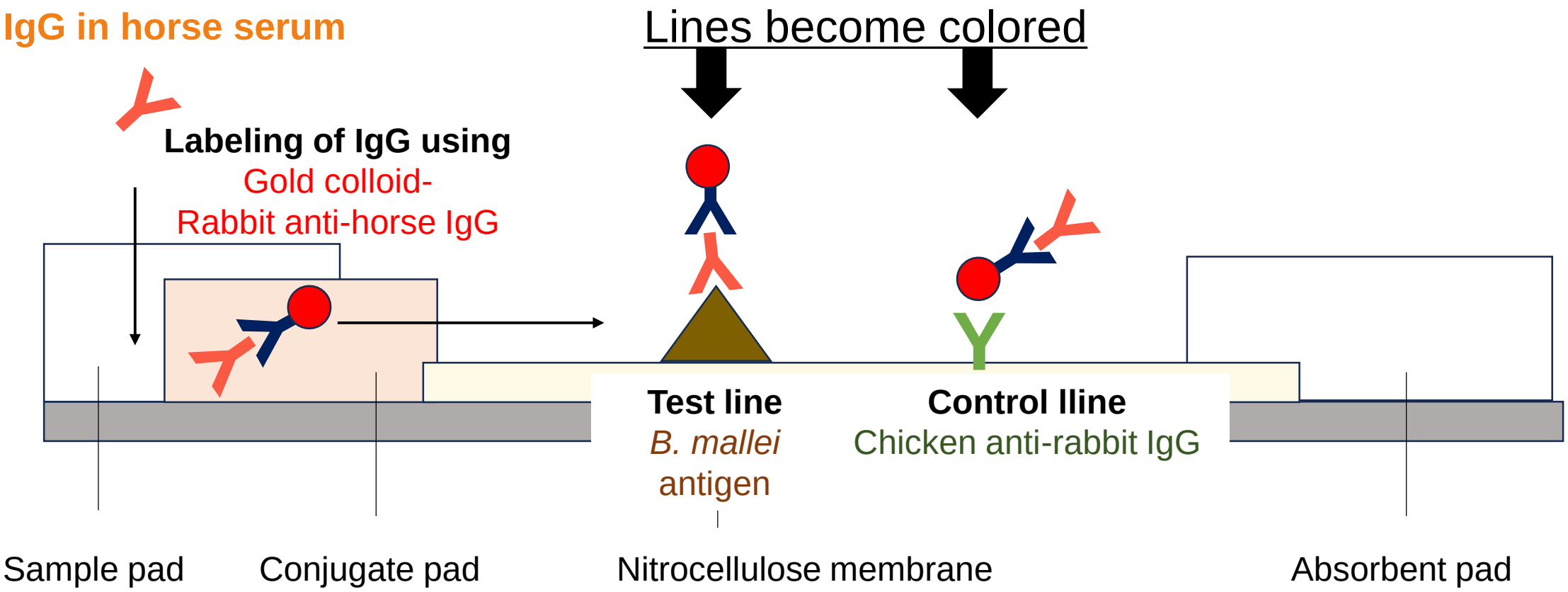
August 9, 2024

Prepared **English manual for making and using the dry LAMP kits**, composed of

- Instruction of Pipejet Nanodispenser “BioSpot” for making dry-LAMP kit, October 26’ 2023
- Protocol on how to prepare in-house reagents for LAMP method, July 13’ 2018
- Composition of LAMP solution, Primer information and Reagent information

PDM 1.5 A prototype ICT was constructed for serodiagnosis of glanders (2020-2023)

Detecting *anti-B. mallei* IgG in horse sera



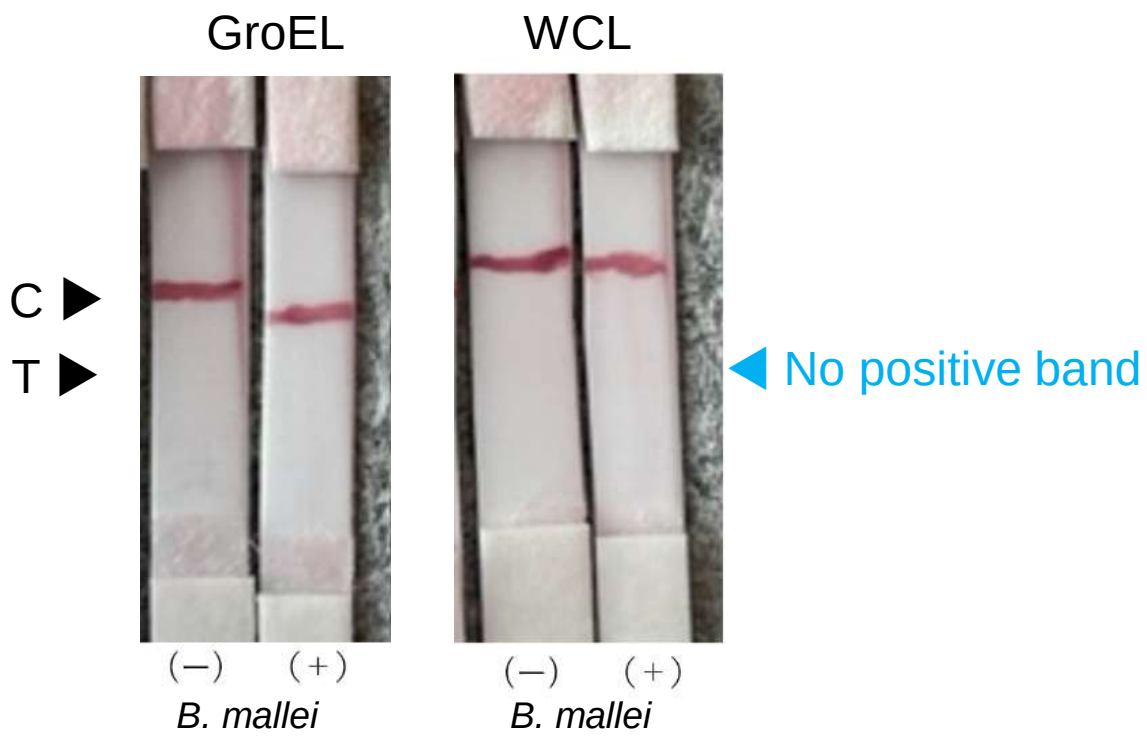
PDM 1.5 A prototype ICT was constructed for serodiagnosis of glanders (2020-2023)

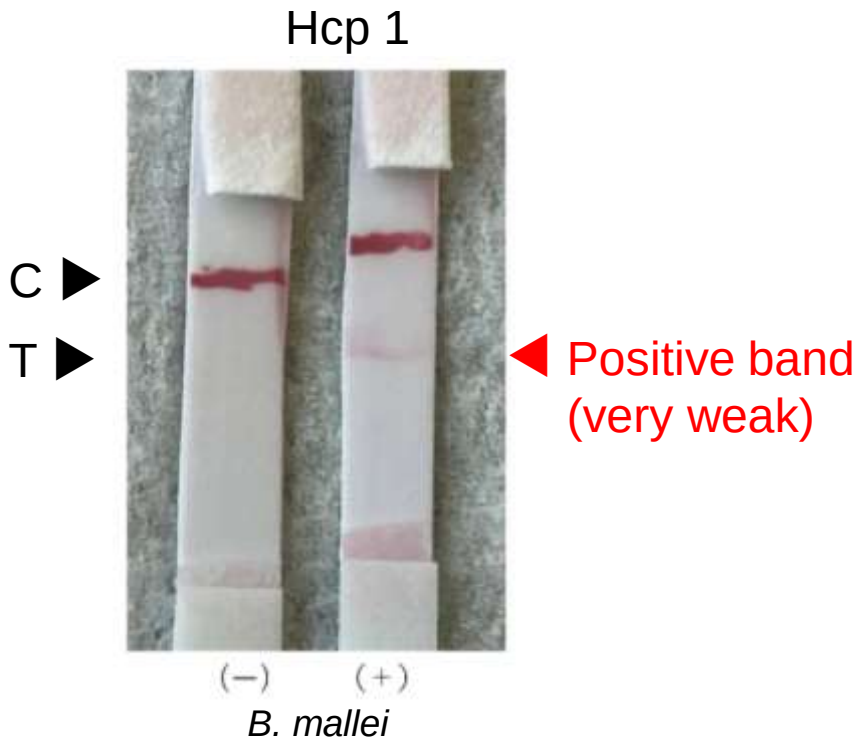
B. mallei antigens applied to the test line

- **GroEL**
- **Hcp 1**
- Whole cell lysate (**WCL**)

Hcp1-ICT	Sensitivity(%)	Specificity(%)
	93.6	100

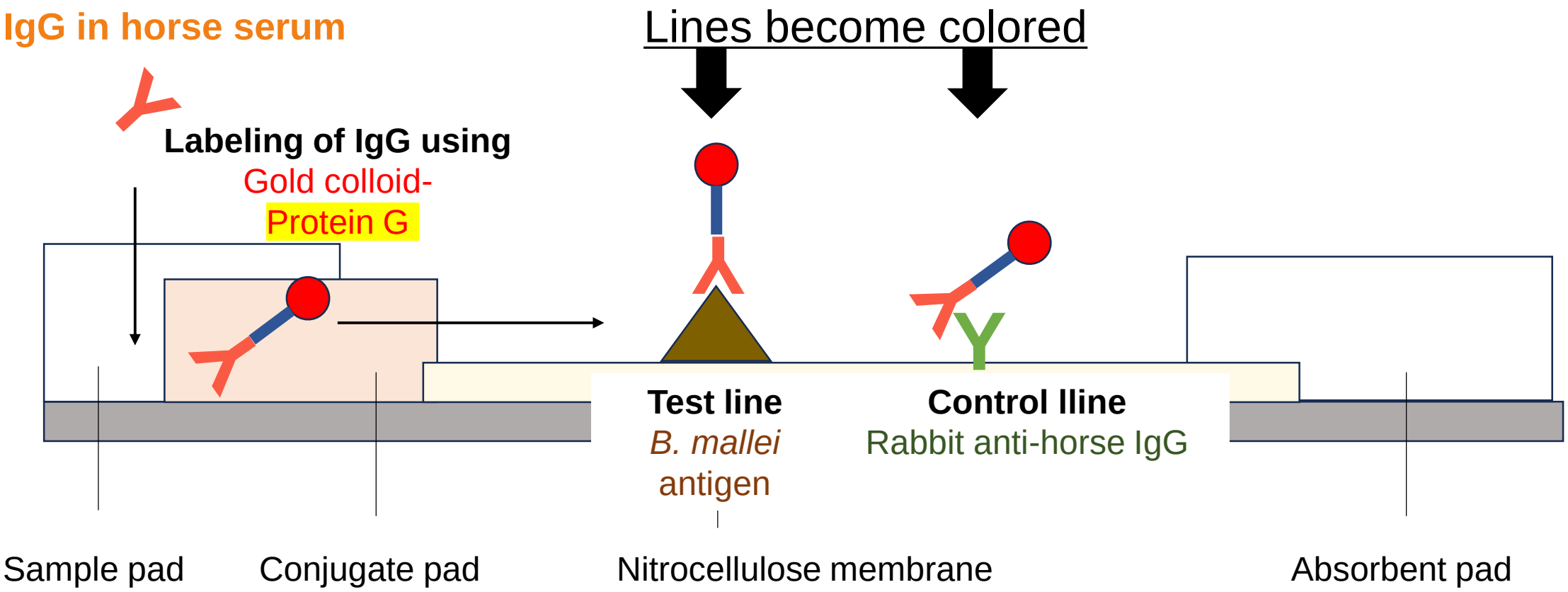
***** 46 *B. mallei*-infected and 50 uninfected horse serum samples were analyzed.





PDM 1.5 Improvement of ICT (2023-2024)

Detecting *anti-B. mallei* IgG in horse sera

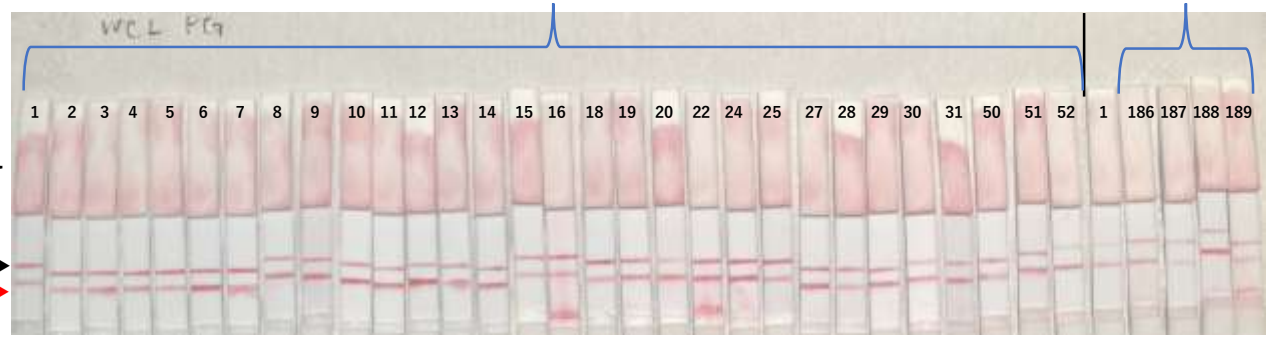


PDM 1.5 Improvement of ICT (2023-2024)

WCL-ICT
B. mallei-Infected horse sera

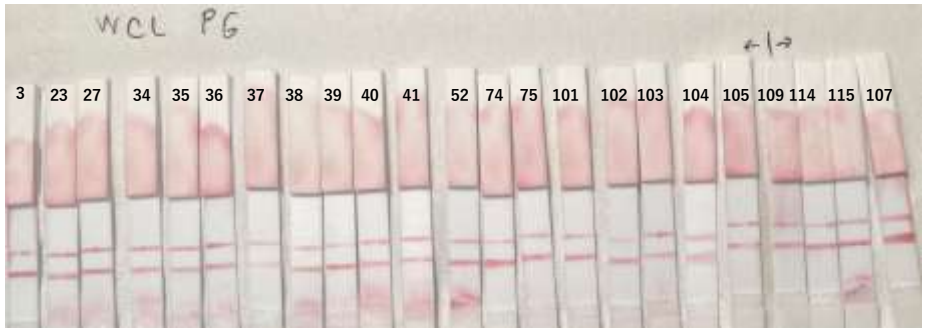
Risk-based

2019 2018



Strong positive bands

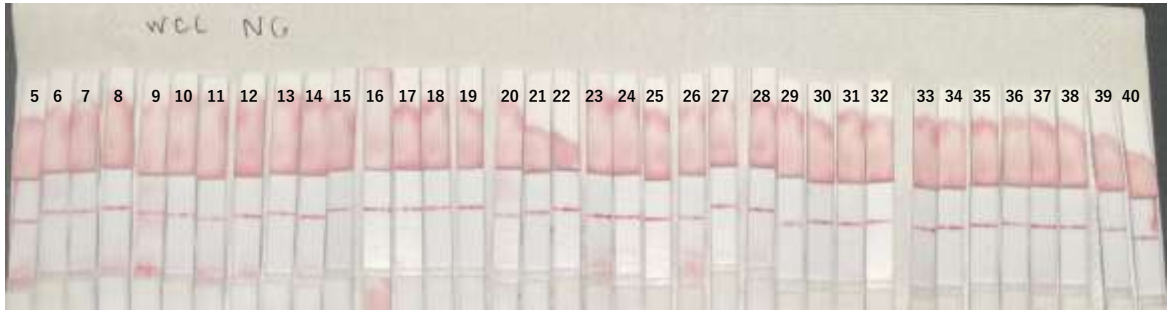
2023



WCL-ICT uninfected sera

2018

2023



WCL-ICT & Hcp1-ICT	Sensitivity(%)	Specificity(%)
	100	100

***** 42 *B. mallei*-infected and 86 uninfected horse serum samples were analyzed.

GroEL-ICT	Sensitivity(%)	Specificity(%)
	92.9	97.7

***** 42 *B. mallei*-infected and 86 uninfected horse serum samples were analyzed.

PDM 1.5 Production of ICT kits using jet dispenser (2023-2024)

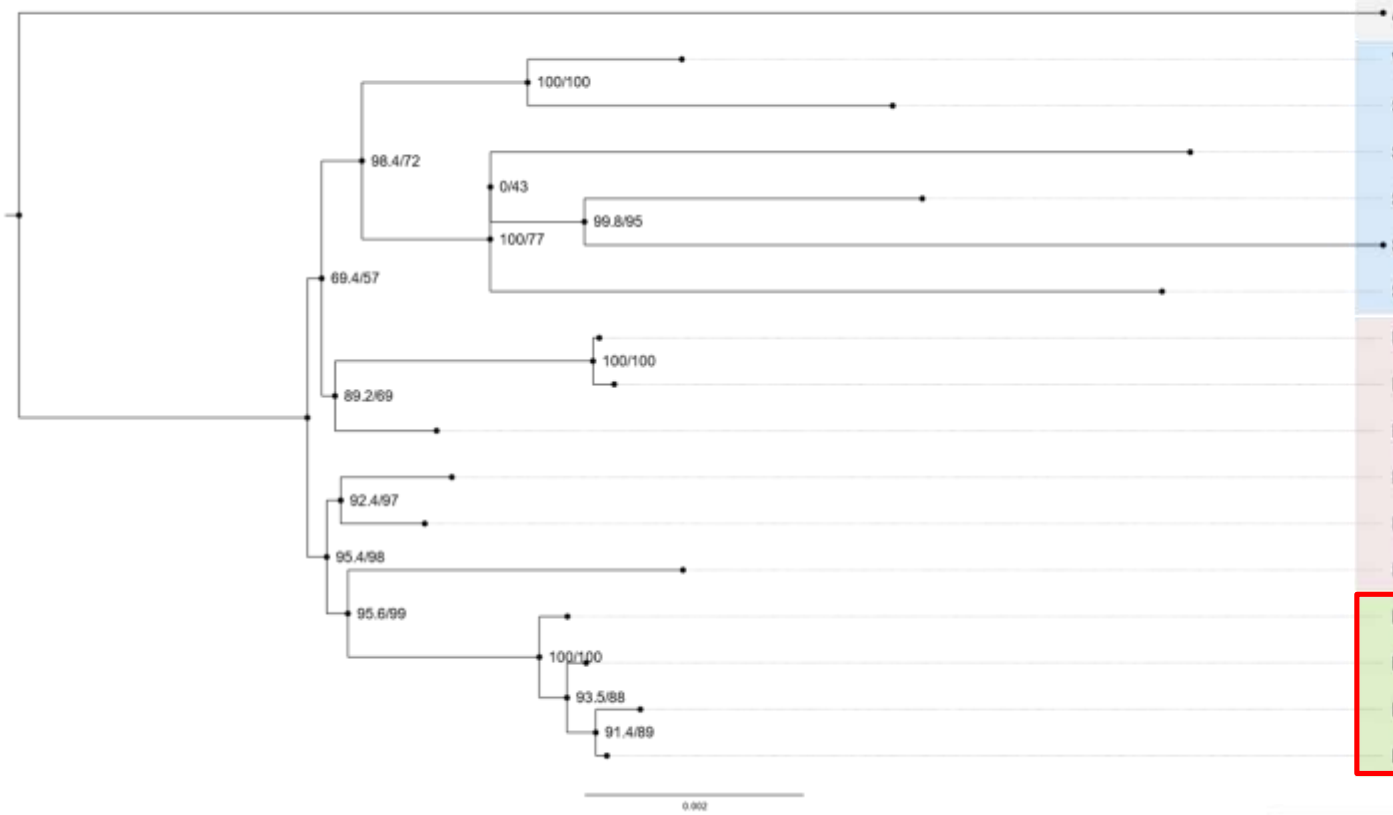
- ✓ Development of the ICT kit has been finished.
- ✓ This kit has diagnostic performance comparable to that of indirect ELISA and is considered to be very useful for serodiagnosis of glanders in Mongolia

I plan to send this equipment to IVM

Jet dispenser

Lamination module

PDM 3.3.4 NGS analysis of Mongolian *B. mallei* isolates (2022-2023)



	Country	Year	Animal
ATCC23344 (Reference)			
V-120	Russia	1985	Horse
SCPM-O-B-7146	Russia	-	-
SCPM-O-B-4684	Russia	-	-
SCPM-O-B-4686	Russia	-	-
SCPM-O-B-4682	Russia	-	-
SCPM-O-B-4683	Russia	-	-
BM-7	China	-	Human
BM-8	China	-	Human
BM-4	China	-	Human
BM-6	China	-	Human
BM-5	China	-	Human
BM-1	China	-	Human
Mongolia No.2	Mongolia	2022	Horse
Mongolia No.1	Mongolia	2022	Horse
Mongolia No.3	Mongolia	2019	Horse
Mongolia No.4	Mongolia	2019	Horse

- : Missing data

- ✓ The Mongolian isolates were located within the L3B1 cluster, and in a unique subbranch
- ✓ Specific SNP markers unique to the Mongolian strains were identified

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

First molecular characterization of *Burkholderia mallei* strains isolated from horses in Mongolia

Yoshiki Ichikawa [a], Liushiqi Borjigin [b], Batchuluun Enkhtuul [c], Ochirbat Khurtsbaatar [c], Keisuke Aoshima [a], Atsushi Kobayashi [d], Vanaabaatar Batbaatar [c], Takashi Kimura [a,*]

[a] Laboratory of Comparative Pathology, Department of Clinical Sciences, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, Japan

Papers on glanders published from FVM, HU

PDM 3.3

1. Ochbayar E, Khurtsbaatar O, Ulziisaikhan G, Batbold Ts, Baatarjargal, **Batbaatar V**, Aoshima K, Kobayashi A, **Kimura T**. Seroprevalence of equine glanders in horses in the central and eastern parts of Mongolia. *J Vet Med Sci*. 2020, 82(9), 1247-1252, doi: 10.1292/jvms.20-0219.
2. Ochbayar E, Baatarjargal P, Khurtsbaatar O, Altanchimeg A, **Batbaatar V**, Aoshima K, Kobayashi A, **Kimura T**. Pathological and Immunohistochemical Analyses of Naturally Occurring Equine Glanders Using an Anti-BpaB Antibody. *Vet Pathol*. 2020, 57(6), 807-811, doi: 10.1177/0300985820953422.
3. Ichikawa Y, **Borjigin L**, Enkhtuul B, Khurtsbaatar O, Aoshima K, Kobayashi A, **Batbaatar V**, **Kimura T**. First molecular characterization of *Burkholderia mallei* strains isolated from horses in Mongolia. *Infect Genet Evol*. 2024,123,105616. doi: 10.1016/j.meegid.2024.105616.

PDM 1.3

1. Nakase M, Thapa J, **Batbaatar V**, Khurtsbaatar O, Enkhtuul B, Unenbat J, Lkham B, Fujita S, Koshikawa A, Tuanyok A, Saechan V, Higashi H, Hayashida K, **Suzuki Y**, Nakajima C, **Kimura T**. A novel ready-to-use loop-mediated isothermal amplification (LAMP) method for detection of *Burkholderia mallei* and *B. pseudomallei. BMC Microbiol*. 2025, 25(1):36. doi: 10.1186/s12866-024-03737-z.

PDM 1.4

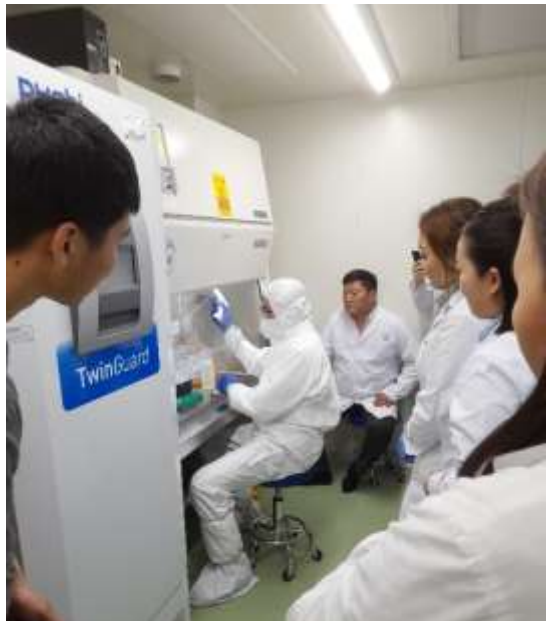
- Currently, one paper on indirect ELISA is being submitted.
- A paper on ICT is planned to be submitted soon.

Procurement of equipment (R/D Annex 8)

- All materials and equipment listed in R/D Annex 8 have been transported to NCCD and IVM.
- The BSL-3 facility will be installed in Room 304 of the IVM Research Building in April 2024 and handed over on the 21st of the same month.

Handover ceremony on April 30, 2024

Mongolian national television coverage (reported as news during prime time on the day)



Demonstration of how to use

Certification of BSL-3 trainer

- ✓ Created an English SOP version 0, with Appendix I-V and Supplementary volume "Protocol for pathogen inactivation“
- ✓ Created educational training materials (Ppt) for those who wish to use the facility

Future outlook

Regarding the activities on glanders

- The development of rapid diagnostic kits planned in PDM has been completed, but in addition, a production system for ICT kits will be established in IVM.

Others

- For both tuberculosis and glanders, more activities are needed toward PDM Output 4-2 and 4-3 (risk assessment as a zoonotic disease).

JICA PDM

Narrative Summary	Objectively Verifiable Indicators	Means of Verification
Overall Goal **Optimized activities with scientific evidences for the control of tuberculosis and glanders are executed in Mongolia.**	1. Specific activities are being implemented for human tuberculosis control, human glanders control (as needed basis), livestock TB control and livestock glanders control in accordance with the control programs or equivalent documents revised or newly-developed based on the scientific evidence. 2. Activities of the Japan-Mongolia One-Health approach platform for zoonosis control are implemented continuously. 3. *<An outcome-level indicator, which can explain the improvement of epidemic situation of zoonotic tuberculosis, shall be determined by six(6) month before the end of the project period.>* 4. *<An outcome-level indicator, which can explain the improvement of epidemic situation of zoonotic glanders, shall be determined by six(6) month before the end of the project period.>*	（1） Revised tuberculosis control program an/or equivalent document(s) （2） Revised livestock infectious disease control program an/or equivalent document(s) （3） Annual Report of the Ministry of Health （4） Annual Report of the Ministry of Food, Agriculture and Light Industry
Project Purpose **An *One-Health* zoonotic disease research base is established in Mongolia for realizing the scientific evidence-based control of tuberculosis and glanders.**	1. By January 2025, practical discussions are commenced with Mongolian authorities concerned for revising the program, guidelines and/or equivalent documents of tuberculosis control on the basis of the novel findings and research outcomes of the Project. 2. By January 2025, practical discussions are commenced with Mongolian authorities concerned for newly-developing and/or revising the program, guidelines and/or equivalent documents of livestock infectious disease control (including tuberculosis and glanders) on the basis of the novel findings and research outcomes of the Project. 3. By 6 months before the end of the project period, at least three (3) research papers, of which the first author is a Mongolian researcher (or comparable responsibility with first author), are published in peer-reviewed scientific journals for each research subject.	(1) Experts' project reports (2) Minutes of the Joint Coordinating Committee (JCC) (3) Handouts and minutes of the Scientific Meetings (4) Other project documents

Need to be done by the end

This project has been evaluated not only by JICA but also by AMED.
I have received the following comments from AMED's Project Supervisor/Officer at the meeting on July 31' 2024.

- Based on the scientific evidence of this project, it is important to make recommendations to the Mongolian government on how to eliminate equine glanders.

- Regarding animal tuberculosis, it would be good to move towards declaring Mongolia as an *M. bovis*-free country to WOAH. Please make recommendations to the Mongolian government on how to achieve this.

- It is important to implement the results of the project. Please be mindful to develop diagnostic guidelines, including the use of kits, and submit them to the Mongolian government.

JICA PDM

Activities after the project ends

Narrative Summary	Objectively Verifiable Indicators	Means of Verification
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Future image of the Platform to be established by the Project

1. Period of implementation of the Project

Joint Coordinating Committee (JCC)

1. Chairperson(s):
Project Director; MOH and/or
Project Co-directors; MECSS and GAVS

2. Project Team Members:
Project Managers: NCCD and IVM
MOH, DPMT, MECSS, MOFALI, and GAVS

Project Technical Committee (TC)

NCCD, IVM, Hokkaido Univ. and Research Institute of Tuberculosis (RIT)

JICA Mongolia Office and Misson
Other concerned personnel, when necessary.

3. Observing Members
MOF, STF, MULS, SVM, SCVL, UVO,
NCZD of MOH, Embassy of Japan in Mongolia, AMED and Other stakeholders appointed by the Chairperson

2. After completion of the Project

Platform for One-Health approach-based infectious disease control

1. Chairperson(s):
- Leader; MOH and/or
- Co-Leaders; MECSS and GAVS

2. Members:
- Technical Committee Managers: NCCD and IVM
- MOH, DPMT, MECSS, MOFALI, and GAVS

Technical Committee (TC)

- NCCD and IVM

Join?

MOF, STF, MULS, SVM, SCVL, UVO, NCZD of MOH, Embassy of Japan in Mongolia, AMED and
Other stakeholders appointed by the Chairperson

3. Advisers
Hokkaido Univ., RIT, Others (OIE, WHO, FAO and other Japanese Univ. etc)

* Detail contents, such structure, members and so on will be considered during implementing the Project.

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Platform for One-Health approach-based infectious disease control

1. Chairperson(s): Same as JCC
- Leader; MOH and/or
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2. Members: Similar to JCC, but do not include Japan side
- Technical Committee Managers: NCCD and IVM
- MOH, DPMT, MECSS, MOFALI, and GAVS

Technical Committee (TC)
- **NCCD and IVM**

Join?

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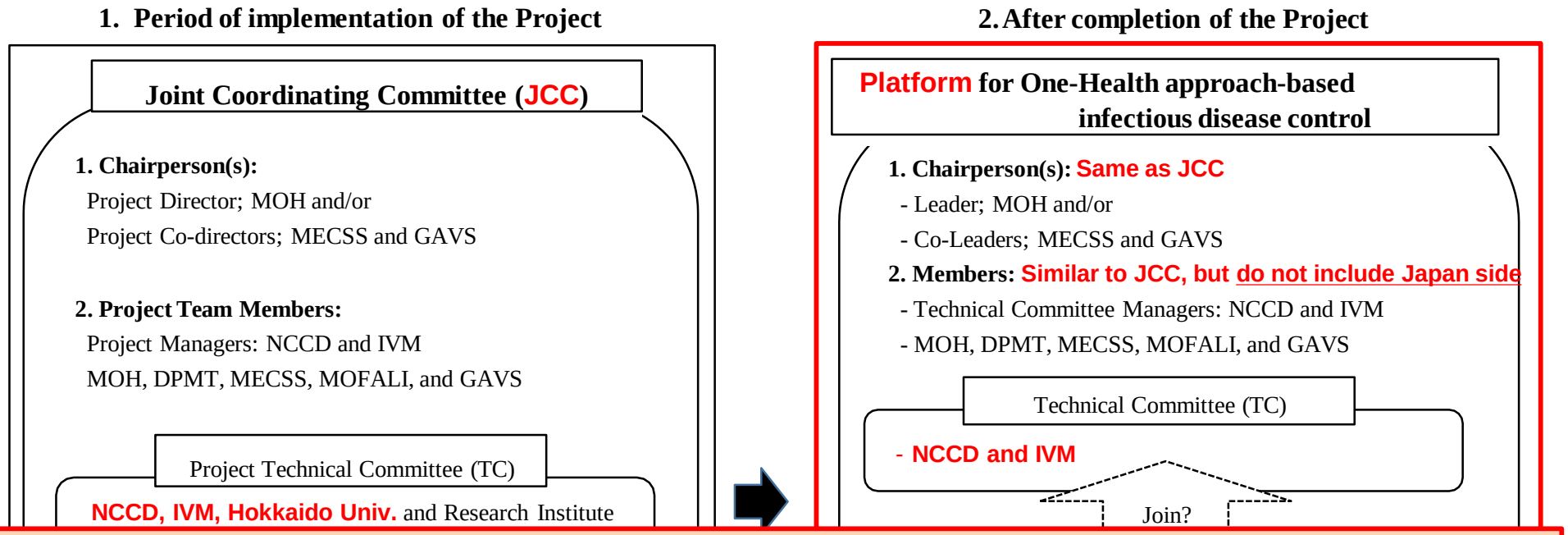

Key Points

- This overall goal, including the platform was proposed by JICA and agreed upon.
- Platform is not a system, but a committee like JCC.
- It is important to have the platform in place before the end of the current SATREPS project.
- This platform needs to continue (at least for 3 years) even if the directors of MOH, NCCD, and IVM are changed, or even if the head of the TB lab at NCCD or the head of the infectious disease and immunology lab at IVM are changed.

Thank you for your attention.