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

Development of immunochromatography as a simple serodiagnostic method for glanders

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Development of simple diagnostic methods for glanders

by the FVM, Hokkaido University

Dry-LAMP method

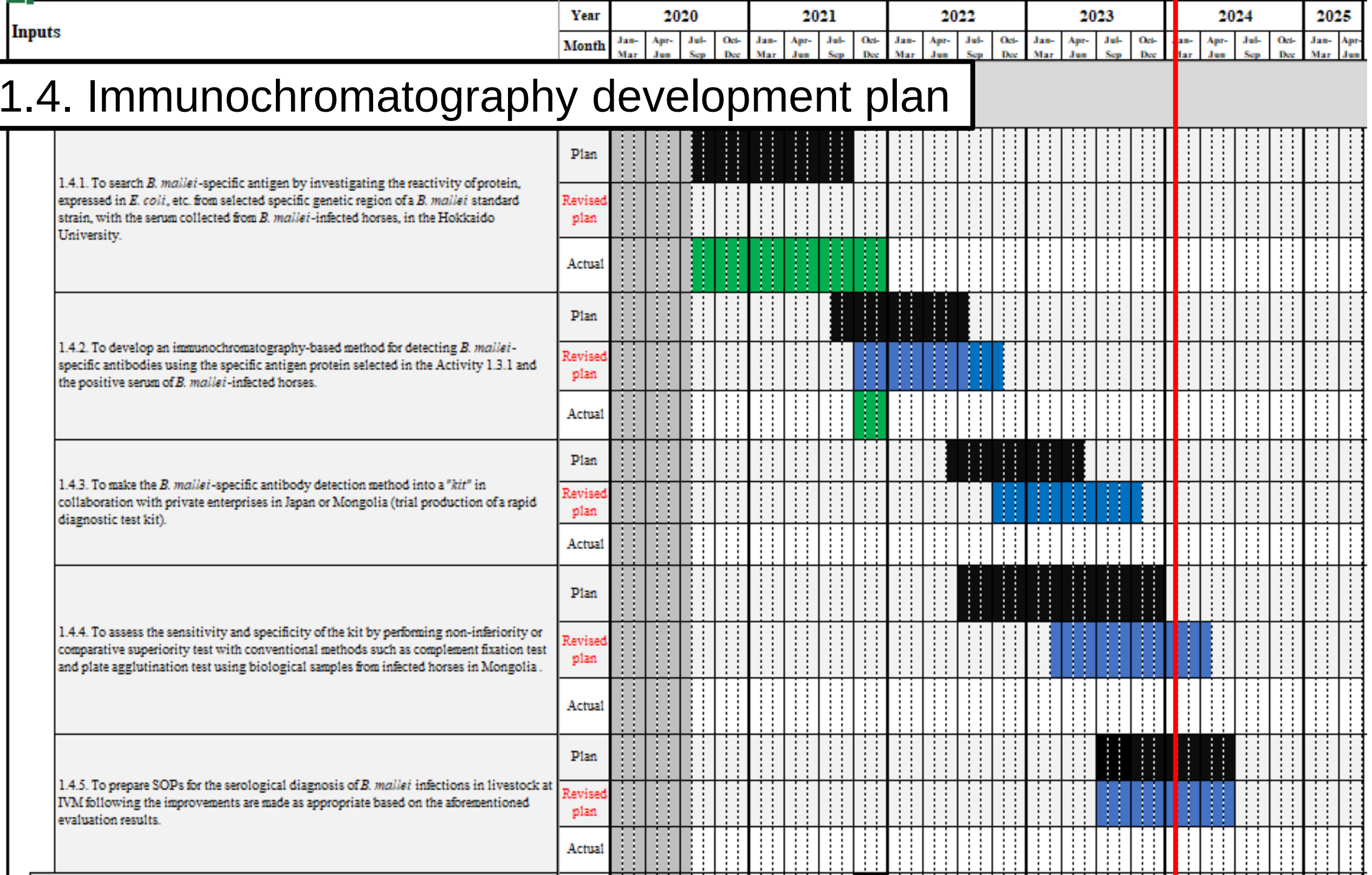
- 1.3. Development of a LAMP-based Rapid diagnostic Method (Test Kit) for *B. mallei* infection
- 1.5. To establish production systems for the genetic diagnostic kits described above by introducing Ink-jet printers into NCCD

Finished

Immunochromatography

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

PO 1.4. Immunochromatography development plan



Diagnosis of Glanders

Conventional methods

Complement fixation test (CFT)	Recommended by WOAH, Highly sensitive High false positive rate, Takes time to make diagnosis Requires rigorous quality control of kit components
Mallein reaction	Once widely used, May give inconclusive results Against animal welfare, Inducing false positive for CFT
Bacterial isolation	Gold standard for disease identification Requires several days and biosafety laboratory
Rose Bengal test (Plate agglutination test)	Low sensitivity and specificity

Recently developed methods

ELISA, Western-blot:	Test (Kits) are expensive Requires equipment

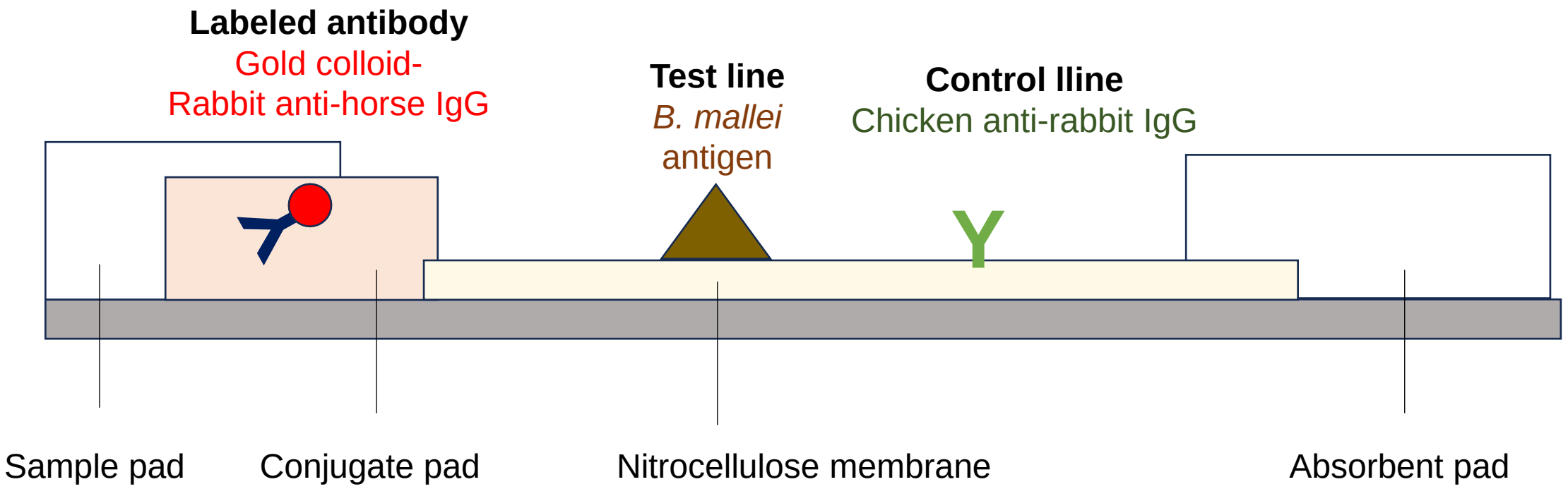
Need for a highly accurate, rapid and inexpensive diagnostic method

Immonochromatographic test (ICT)

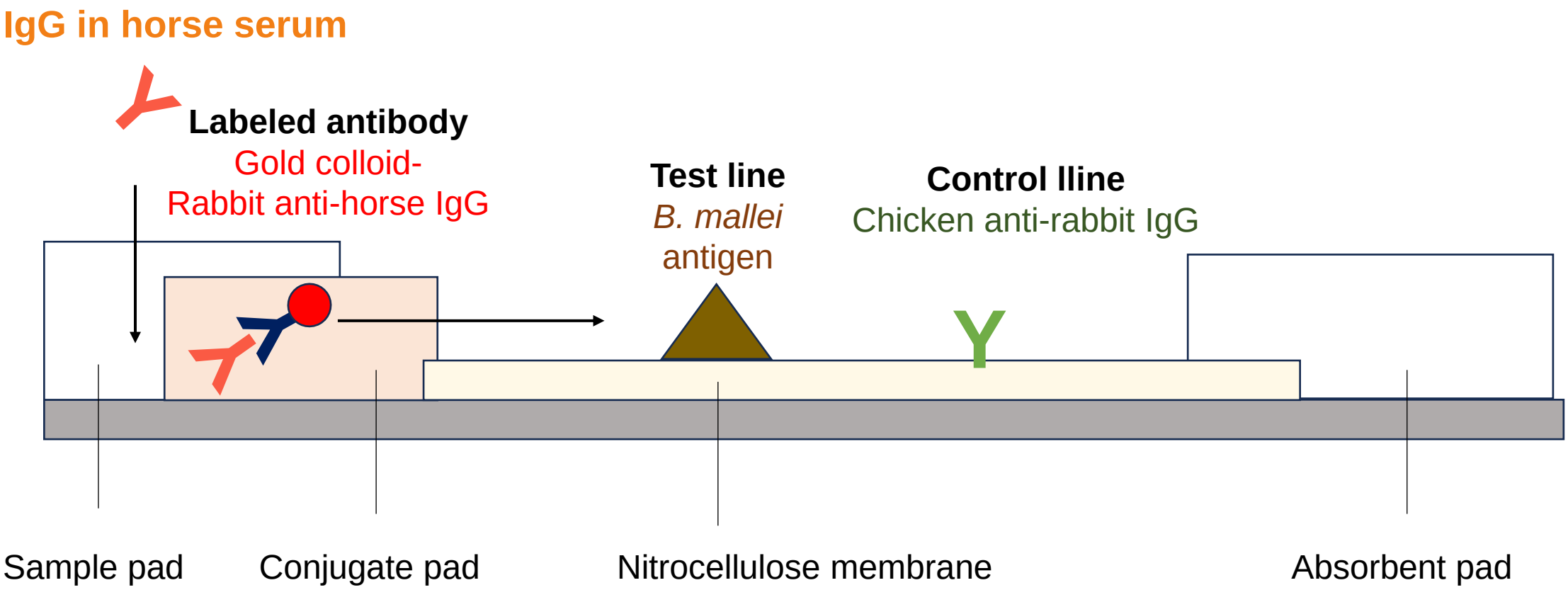
- Immunoassay using capillary phenomenon

Procedure	Add a few drops of sample solution and leave it	Simple
Time required	15 to 20 minutes	Rapid
Judgment	Visual inspection (no equipment required)	Low cost

Configuration of ICT

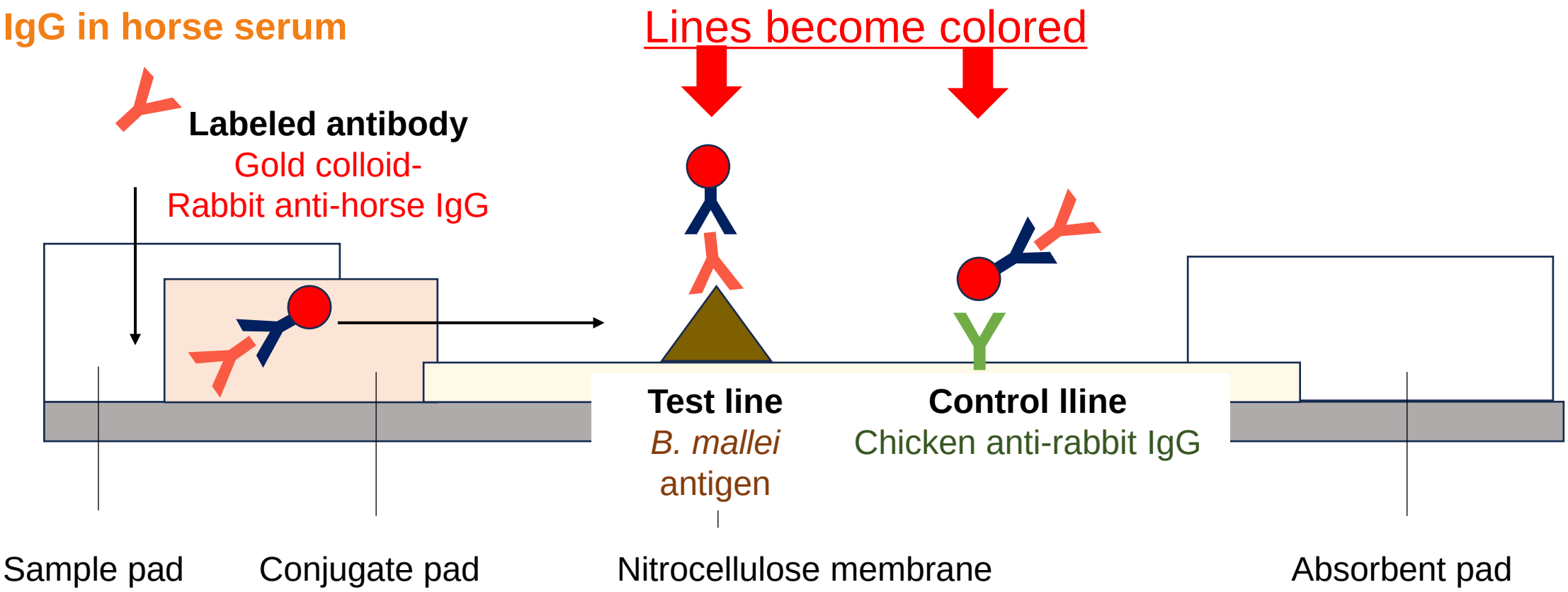


Configuration of ICT



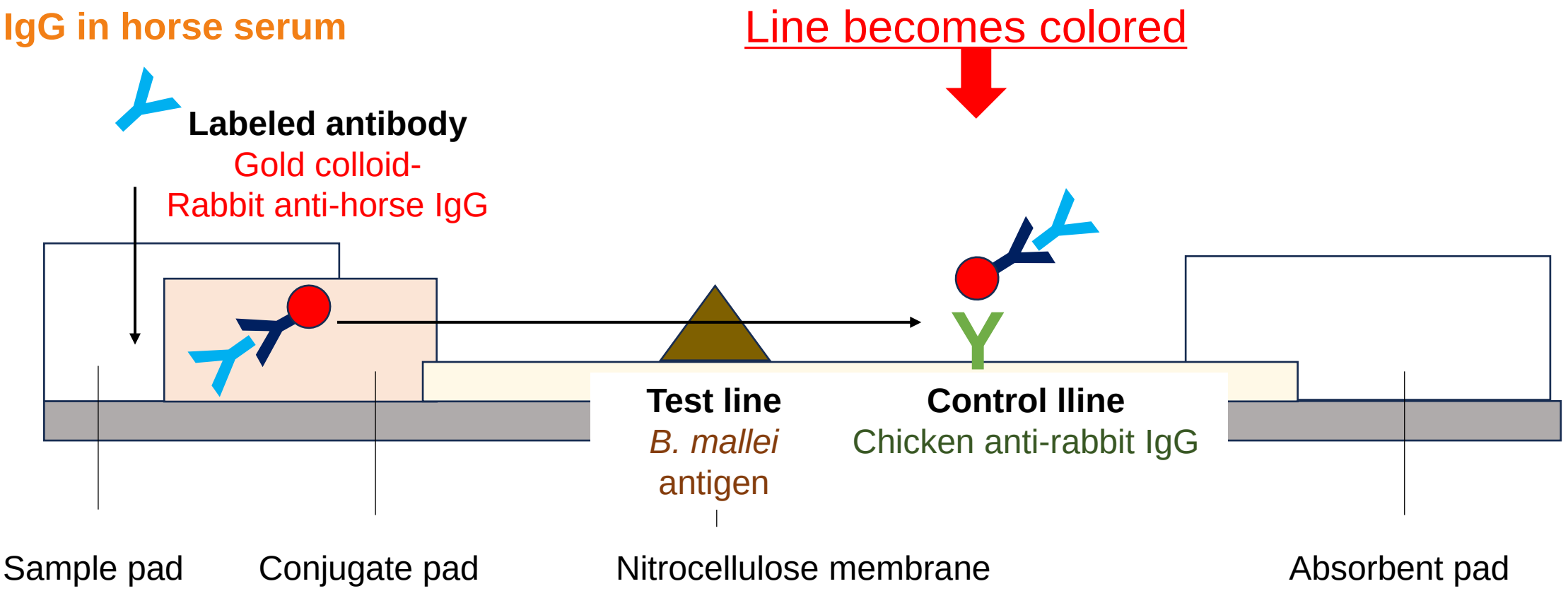
Configuration of ICT

Infected horse serum (with *anti-B. mallei* IgG)



Configuration of ICT

Uninfected horse serum (without *anti-B. mallei* IgG)



Comparison of reactivity of *B. mallei* antigens

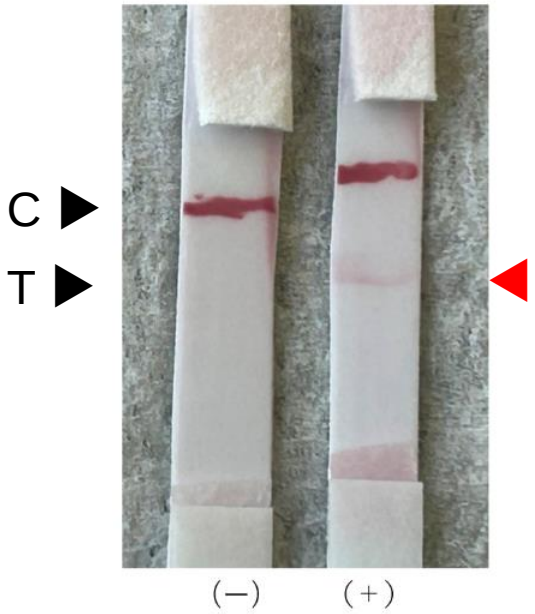
Candidate of B. mallei antigen applied to the test line

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

Indirect ELISA Ichikawa *et al.*, submitted

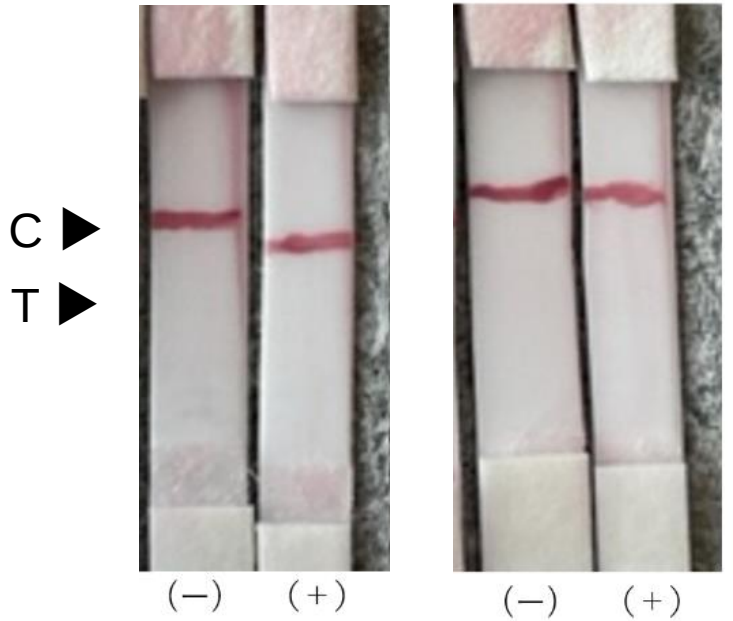
	Sensitivity	Specificity (%)
GroEL	100	92
Hcp 1	97.3	100
WCL	100	100

Hcp 1



Positive band

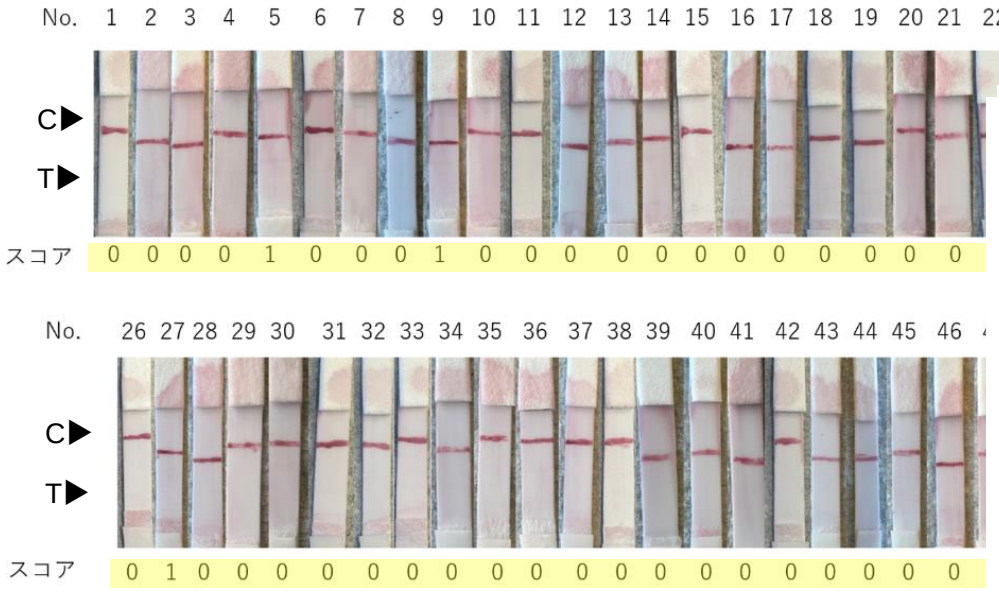
GroEL WCL



No positive band

Hcp1-ICT: sensitivity and specificity

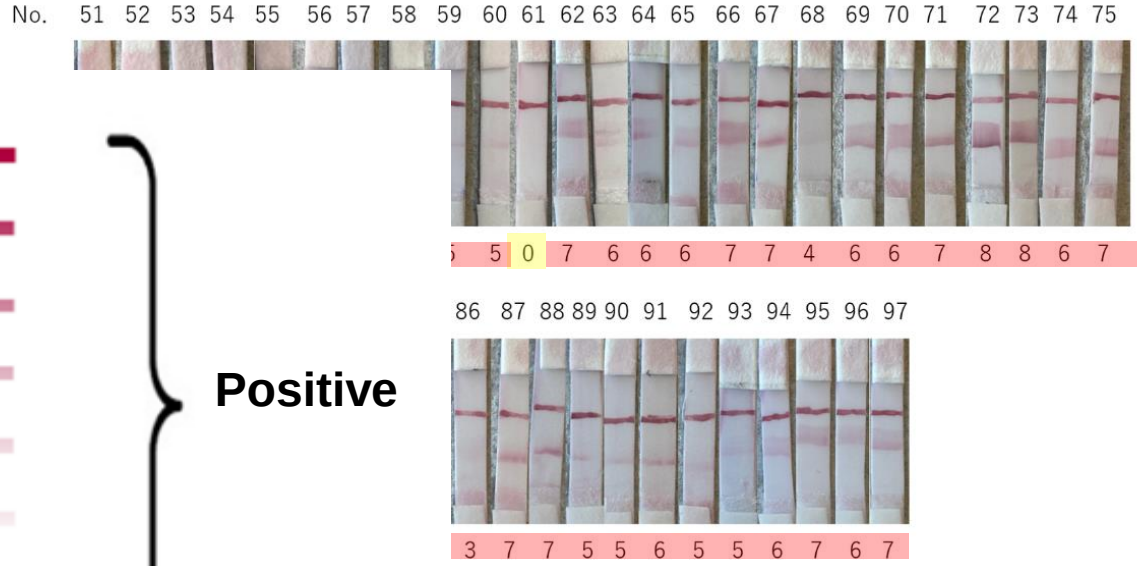
Uninfected horse serum CFT(-), Mallein(-), Clinical signs(-)



All uninfected horse sera (n=50) tested neg

Hcp1-ICT

***B. mallei*-infected horse serum** CFT(+), Mallein(+), Clinical signs(+)



Positive

Negative

cted with *B. mallei* (n=47), 44 itive.

Discussion

Hcp1-ICT prepared in this study has high diagnostic accuracy, suggesting that it may be useful as a rapid and simple serological diagnosis comparable to the conventional diagnosic method for glanders

However,

- Three of the 47 infected horse serum samples tested negative
- GroEL-ICT and WCL-ICT did not show positive band against infected horse serum

What we need to work on;

- Increasing the sensitivity of Hcp1-ICT so that a band appears even with a small amount of anti-*B. mallei* IgG in the blood
- Making GroEL-ICT and WCL-ICT that can be used for diagnosis

Opinion about the project as a whole

- It is necessary to proceed with risk assessment of animal-to-human transmission of tuberculosis and glanders.
- In particular, little analysis has been conducted regarding the risk of *B. mallei* transmission from animals to humans.
- In order to proceed with this matter efficiently, I would like to propose a revision to PDM2.5. "Epidemiological evaluation of the epidemics of B. mallei Infection in human“ (please refer to the next slide)

- We have developed novel diagnostic methods; however, some reagents appear to be difficult to obtain in Ulaanbaatar. We would appreciate your cooperation in this regard.

	Current PDM	Revision proposal	Reasons
2.5.1	NCCDにおいて原因病原体が特定されていないヒト感染性肺炎患者から得られた生体試料（喀痰ないし咽頭拭い液）を収集し、IVMに移送する。To collect the biological samples (sputum and/or throat swab) obtained from patients with human infectious pneumonitis for whom no causative agent has been identified in the NCCD, followed by transferring them to IVM.	GAVS等の機関が所有している鼻疽罹患馬を飼育している牧夫の情報をNCCDに供与し、該当する牧夫と必要であればその関係者より血液を採取する。To collect blood from the herdsmen and their related persons who raise B. mallei-infected horses by providing information on the herdsmen who raise B. mallei-infected horses owned by organizations such as GAVS to NCCD.	現在の PDM の手法を使用した場合、鼻疽馬と接触した人々を選択的に分析することは困難であるため Because using current PDM method, it is difficult to selectively analyze people who have had contact with glandrous horses.
2.5.2	収集した生体試料をIVMにおいて活動1．2で開発した遺伝子検出法でスクリーニングし、B. malleiヒト感染例の有無を評価する。To evaluate the presence of human cases of B. mallei infection in IVM by screening them with the gene detection method developed in Activity 1.2.	収集した血液をIVMにおいてスクリーニングし、B. malleiヒト感染例の有無を評価する。To evaluate the presence of human infection with B. mallei by screening the collected human blood in IVM.	