World Organisation for Animal Health

中国兽医药品监察所
China Institute of Veterinary Drug Control
国家动物布鲁氏菌病参考实验室
National Reference Laboratory for Animal Brucellosis

Proficiency Testing and Lab Capacity Building about animal Brucellosis Diagnostic Methods for WOAH Asia-Pacific Members

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2nd WOAH Regional Training Workshop on Brucellosis Diagnosis

Beijing, P.R. China, 26 - 29 May 2026

Contents

01 Introduction

The Need for Accurate Diagnosis

01. Control & Eradication

Scientific and accurate diagnosis is the cornerstone for effective prevention, control, and eradication strategies.

02. Diagnostic Complexity

No single test is perfect. Diagnosis often requires the **integration and comprehensive analysis of multiple test results.**

01 Introduction

Challenges and gaps

Technical & Infrastructure

- ✓ Variable quality of reagents/kits
- ✓ Significant discrepancies exist between official reports and confirmatory diagnostic evidence.
- ✓ **Low adherence to WOAH standards**

Human Resource Gaps

- ✓ Insufficiently trained laboratory and veterinary personnel
- ✓ Lack of laboratory capacity
- ✓ Inconsistent diagnostic methods

QA & PT Participation

- ✓ Low awareness of PT benefits
- ✓ Absence of national PT schemes in many countries.

Systemic & Policy Issues

- ✓ Insufficient sustainable funding
- ✓ fragmented disease surveillance networks

01 Introduction

The Importance of Proficiency Testing (PT)

Goals of PT

Assess Performance

Objectively assesses individual lab performance

Identify Errors

Identifies technical gaps and areas for improvement

Standardize Practices

Unify standard testing protocols across the network

Ensure Comparability

Ensure Comparability and consistent results across the WOAH Asia-Pacific region

Build Confidence

Provide **objective national surveillance data**

01 Introduction

The PT Process: Step-by-Step

01 Sample Preparation & Distribution

A PT provider prepares coded, blind serum samples with known values and distributes them to participating labs.

02 Testing by Participating Labs

Labs test the samples using their routine procedures, unaware of the expected results.

03 Result Submission

Labs report their results to the PT provider by a specified deadline.

04 Data Analysis & Reporting

The provider collates results and performs statistical analysis, generating a comprehensive performance report.

05 Feedback & Corrective Action

Labs receive reports; those with unsatisfactory results investigate root causes and implement corrective actions.

02 WOAH Recommended Official Diagnostic Methods

- **Pathogenic Methods**(Direct Detection)

Identify the *Brucella* organism directly to confirm bacterial presence in samples.

Examples: Bacterial Culture, PCR，etc.

- **Serological Methods**(Indirect Detection)

Detect host antibodies produced in response to *Brucella* infection.

Examples: RBT、MRT、CFT, ELISA, FPA, etc.

02 WOAH Recommended Official Diagnostic Methods

➢ Pathogenic Methods: Bacterial Culture (Gold Standard)

Principle

The isolation and definitive identification of *Brucella* bacteria directly from clinical samples (e.g., blood, bone marrow).

Standard Procedure

1.**Preparation:**Inoculate samples onto selective agar media.

2.**Incubation:**37°C in 5-10% CO_2 for up to 7 days.

3.**ID:**Morphology + Biochemical tests; MALDI-TOF confirmation.

Key Advantages

- **Definitive Diagnosis:** Absolute proof of infection.
- **Strain Typing:** Critical for epidemiological tracking.

⚠ Limitations

• Time-consuming (days/weeks) • High-hazard (BSL-3 required) • Dependent on highly skilled technicians and specialized equipment.

02 WOAH Recommended Official Diagnostic Methods

➢ Pathogenic Methods: Molecular Methods (e.g. PCR)

Key Advantages

- **Rapid:** Fast (hours vs. days for culture).
- **High Performance:** High sensitivity/specificity.

Limitations & Challenges

- **Contamination Risk:** False positives from cross-contamination.

PCR (Polymerase Chain Reaction)

Key Assays: Conventional (genus-level), AMOS-PCR (species), **Bruce-Ladder** (typing all species).

Quantitative Real-time PCR (q-PCR)

Pros: Enhanced sensitivity/specificity, **quantitative data** on pathogen load.

Cons: Requires costly equipment and specialized technical training.

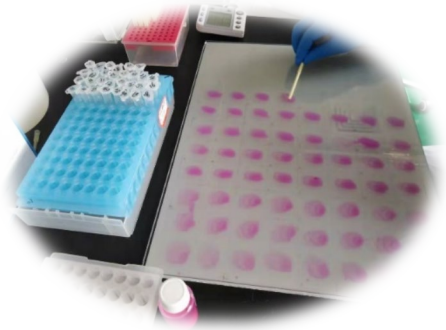
LAMP (Loop-mediated Isothermal Amplification)

Advantages: Simple operation, **no thermal cycler required,** ~30 mins.

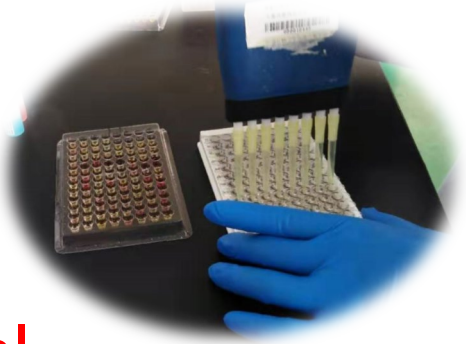
Perfect for **Point of Care (POC)** and resource-limited field applications.

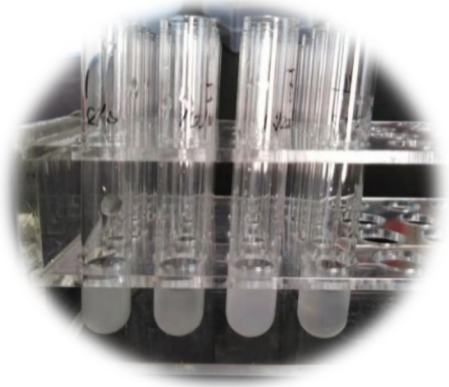
02 WOAH Recommended Official Diagnostic Methods

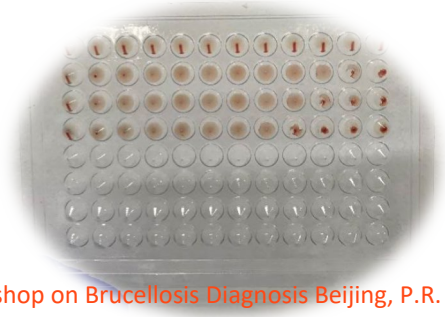
➢ Serological Diagnostic Methods

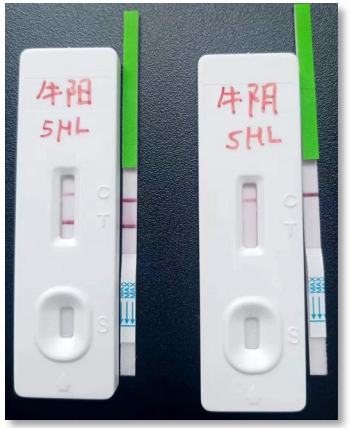


1. Rose bengal test （RBT）
2. Standard tube agglutination test(SAT)
3. Complement fixation test(CFT)
4. iELISA and cELISA
5. Milk ring test （MRT）
6. Fluorescence polarization assay(FPA)
7. Immunochromatographic Assay(ICA)： colloidal gold， Color Gel, Fluorescent microsphere









02 WOAH Recommended Official Diagnostic Methods

➢ Serological Diagnostic Methods

Screening Tests

- **Rose Bengal Test (RBT):**rapid screening.
- **Milk Ring Test (MRT):**herd-level screening in dairy cattle populations.
- **ICA:**Easy-to-use, rapid on-site testing.
- **FPA:**WOAH-recommended high-throughput method.
- **ELISA (i/c):**cELISA achieves high sensitivity (97.9%) and near-perfect specificity (~100%).

Confirmatory Tests

- **CFT & COMPELISA:**gold standard; COMPELISA variant offers enhanced sensitivity.
- **ELISA (i/c):**cELISA achieves high sensitivity (97.9%) and near-perfect specificity (~100%).
- **SAT:**Classic quantitative titration.

Core Challenge: The DIVA Problem

Differentiating infected animals from vaccinated animals (DIVA) is difficult, as current vaccines induce antibodies that cross-react with those from natural infection.

03 Recommendations and Future Perspectives

03 Recommendations and Future Perspectives

➢ Support Recommendations

Enhancing Human Resources & QA

- Conduct targeted **training courses** on diagnosis, QC, and PT .
- Support **"Twinning" projects** .
- Develop accessible **e-learning resources**.
- Establish sustainable, cost-effective **regional PT schemes**.
- Provide **financial support** for PT participation.
- Assist members in **implementing QMS** based on ISO 17025.

Fostering Collaboration

- ◆ Strengthen Surveillance Networks
- ◆ Promote Regional Information Sharing
- ◆ Advocate for "One Health" Approach
- ◆ Mobilize Resources

➢ Recommendations for Member Countries

Strengthen Laboratory Quality Systems

Improve Surveillance & Reporting

Enhance Regional Cooperation

Expand Diagnostic Capacity

Invest in Workforce Development

04 Proficiency Testing (PT) Scheme of RBT

Details refer as Words version

1. Introduction & Scope

2. Regulatory & Scientific Basis

- WOAH
- Chinese Standard: GB/T 18646-2025.

3. Panel Preparation & Shipment (Blind Sample Management)

3.1 Sample Composition

3.2 Blind Processing

4. Test Procedure & Operational Protocol (RBT)

Participants must strictly follow the manual method described the following .

1 **Reagent Prep** pH 3.65 ± 0.05. Stored at 4°C (Do not freeze).
2 **Sample Prep** Bring sera to room temperature.
3 **Dispensing** Place 30µl of serum onto a clean white porcelain/glass plate.
4 **Antigen** Add 30µl of well-mixed RBT antigen adjacent to the serum.
5 **Mixing** Mix thoroughly using a clean stick to create a 2cm diameter zone.
6 **Incubation** Rock the plate gently for 4 minutes at ambient temperature (18-22°C).
7 **Reading** Read immediately in good light.

04 Proficiency Testing (PT) Scheme of RBT

Details refer as Words version

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7	**Reading**	Read immediately in good light.

04 Proficiency Testing (PT) Scheme of RBT

5. Interpretation of Results (Judgment Criteria)

Results are based on agglutination visibility.

6. Reporting & Evaluation

- Participants must submit results using Appendix A within 20 working days.
- Evaluation Scoring:
  - ➢ Satisfactory: 4/4 correct identifications.
  - ➢ Unsatisfactory: <4/4 correct.

Details refer as Words version

Root Cause Analysis (RCA): Unsatisfactory labs must submit an RCA form covering:

- Antigen storage temperature logs (did it freeze?).
- Timer accuracy (Was the 4-minute rock exact?).
- Reading subjectivity (Edge drying misinterpretation).

凝集反应程度 Degree of agglutination reaction	观察结果 Observation	结果解释 Interpretation
++++	出现大的凝集片或颗粒，液体完全透明。 Large agglomerates or particles appear, and the liquid is completely transparent.	抗体阳性 Positive for Brucella antibodies
+++	有明显的凝集颗粒，液体几乎完全透明。 There are visible agglutinated particles, and the liquid is almost completely transparent.	抗体阳性 Positive for Brucella antibodies
++	有较明显的凝集颗粒，液体稍透明。 There are relatively obvious agglutinated particles, and the liquid is slightly transparent.	抗体阳性 Positive for Brucella antibodies
+	稍能见到凝集，液体混浊。 Slight agglutination can be observed, and the liquid is turbid.	抗体阳性 Positive for Brucella antibodies
-	无凝集，液体均匀混浊。 No agglutination, with the liquid being uniformly turbid.	抗体阴性 Negative for Brucella antibodies or No antibodies detected

04 Proficiency Testing (PT) Scheme of RBT

Details refer as Words version

Appendix A

Results Submission Form

Participant Code or Name Date

Test Method: Manual RBT, Antigen Lot No.

Temp/RH ___ °C / ___% Operator:

Vial ID	Final Interpretation (Pos/Neg)
PT-01A	Yes / No
PT-01B	Yes / No
PT-02A	Yes / No
PT-02B	Yes / No

04 Proficiency Testing (PT) Scheme of RBT

Proficiency Testing (PT) Scheme for Brucellosis Diagnosis:

Rose Bengal Test (RBT)

Scheme ID: PT-RBT-2026-INT
Reference Standards: WOAH Terrestrial Manual (Chap. 3.1.4), GB/T 18646-2025
Target: Participants of International Workshop or Trainee

1. Introduction & Scope

- Brucellosis is a major zoonotic disease, and serological surveillance is critical for control programs. The Rose Bengal Plate Test (RBT) is recognized by WOAH as the primary screening test due to its good sensitivity and rapid turnaround .
- This proficiency testing scheme is designed to standardize RBT procedures across international labs. Participants will test a blind panel of 2 sera (including weakly positive threshold samples) to evaluate lab accuracy.

2. Regulatory & Scientific Basis

This scheme strictly follows the methodologies defined in:

- WOAH: Brucellosis (*Brucella abortus, B. melitensis* and *B. suis*) (Chapter 3.1.4) .
- Chinese Standard: GB/T 18646-2025 Diagnostic techniques for animal brucellosis .

3. Panel Preparation & Shipment (Blind Sample Management)

3.1 Sample Composition

- **The panel**: consists of 2 coded vials (0.5ml – 1.0ml each), lyophilized.
- **Content**: Standard Sera panel (Prepared and calibrated by WOAH/FAO/National brucellosis reference laboratory).
- **Profile:** 1 Positives, 1 Negatives.
- **Stability:** Stored at 4°C.

3.2 Blind Processing

All vials will be labeled with random codes (e.g., PT-01 to PT-02). The "Key" (expected results) will be kept confidential by the PT Provider until the submission deadline.

The lyophilized serum sample should be reconstituted by adding 1.0 mL of saline, followed by gentle mixing before use.

Test sample A is diluted as follows: 50 µL of the original suspension is mixed with 450 µL of saline buffer to obtain a 10-fold dilution. Test sample B is then diluted by adding 100 µL of the A suspension to 800 µL of saline buffer, resulting in a 90-fold dilution.

Consequently, the test samples are INT-01A, INT-01B, INT-02A, and INT-02B.

4. Test Procedure & Operational Protocol (RBT)

Participants must strictly follow the manual method described the following .

1) **Reagent Prep** pH 3.65 ± 0.05. Stored at 4°C (Do not freeze).
2) **Sample Prep** After blinding process(Ref 3.2), Bring test sample to room temperature.
3) **Dispensing** Place 30µl of test sample onto a clean white porcelain/glass/plastic plate.
4) **Antigen** Add 30µl of well-mixed RBT antigen adjacent to the serum .
5) **Mixing** Mix thoroughly using a clean stick to create a 2cm diameter zone.
6) **Incubation** Rock the plate gently for 4 minutes at ambient temperature (18-22°C).
7) **Reading** Read immediately in good light.
8) **Result report** **only "positive" or "negative"**

5. Interpretation of Results (Judgment Criteria)

Results are based on agglutination visibility.

凝集反应程度 Degree of agglutination reaction	观察结果 Observation	结果解释 Interpretation
++++	出现大的凝集片或颗粒，液体完全透明。Large agglomerates or particles appear, and the liquid is completely transparent.	抗体阳性 Positive for *Brucella* antibodies
+++	有明显的凝集颗粒，液体几乎完全透明。There are visible agglutinated particles, and the liquid is almost completely transparent.	抗体阳性 Positive for *Brucella* antibodies
++	有较明显的凝集颗粒，液体稍透明。There are relatively obvious agglutinated particles, and the liquid is slightly transparent.	抗体阳性 Positive for *Brucella* antibodies
+	稍能见到凝集，液体混浊。Slight agglutination can be observed, and the liquid is turbid.	抗体阳性 Positive for *Brucella* antibodies
-	无凝集，液体均匀混浊。No agglutination, with the liquid being uniformly turbid.	抗体阴性 Negative for *Brucella* antibodies orNo antibodies detected

6. Reporting & Evaluation

- Participants must submit results using Appendix A within 20 working days.
- Evaluation Scoring:
  - ➢ Satisfactory: 4/4 correct identifications.
  - ➢ Unsatisfactory: not completely (4/4) correct.

Root Cause Analysis (RCA): Unsatisfactory labs must submit an RCA form covering:

- Antigen storage temperature logs (did it freeze?).
- Timer accuracy (Was the 4-minute rock exact?).
- Reading subjectivity (Edge drying misinterpretation).

2

7. Request for Comments

- To further optimize the rationality, international applicability and operability of the protocol, **all participats are sincerely invited to put forward valuable modification opinions and suggestions**.
- **The solicited opinions mainly include but are not limited to:** protocol rationality, operation step optimization, sample setting scheme, standard adaptation, and problems existing in actual international testing application.

Feedback Method: Please feed back written opinions (English) to the organizing unit within the specified feedback period, including modified content, modification basis and optimization suggestions. The organizing unit will summarize and demonstrate all valid opinions, and revise and improve the final official protocol to form a more standardized and international unified brucellosis diagnosis capability verification standard.

Deadline for Comments: [30/05/2026]

Please direct comments to:
Technical Manager: **Dr. Yunhao Hu**
Email: huyhaoo@163.com and CC it to nrlab_ivdc@163.com

Subject: RBT-PT Comments/[Your Lab Code or your Name]

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05 Discussion:Comments or solicited opinions of PT Scheme

Another important issue to consult everyone in order to better facilitate international lab PT and cooperation.

- ✓ **We design a questionnaire table. Please fill in it truthfully based on the actual situation of your country and your institution.**
- ✓ **If possible(especially customs). Our lab is very willing to provide reference agents for international training participant.**
- ✓ **May I ask if it would be possible for your organization to issue a certifying document signed by yourself, as well as provide the relevant results? Please let us know if you encounter any difficulties.**
- ✓ **Also if it is conventient for RRPA or WOAH to provide some additional support documents.**

05 Discussion

Questionnaire

Introduction

To collect technical information on livestock population, brucellosis epidemiology, vaccines, diagnostic reagents, laboratory networks, and willingness to participate in inter-laboratory proficiency testing (PT). All collected data will be only used for academic research, animal disease prevention and control analysis and international technical cooperation research, and will be kept strictly confidential. Please fill in the questionnaire truthfully based on the actual situation of your country and your institution.

Basic Information

1. Your Country/Region: _____
2. Your Affiliated Institution: _____
3. Your Job Position: _____
4. Contact Email: _____

Part 1. National Cattle and Sheep Breeding Scale

Please fill in the annual breeding inventory of cattle and sheep in your country (the latest official statistical data):

1.1 Total national cattle inventory: _____ (heads)

1.2 Total national sheep inventory: _____ (heads)

Part 2. National Brucellosis Epidemic Situation (Zoonotic Brucellosis)

2.1 Is brucellosis a notifiable animal disease in your country?
☐ Yes ☐ No

2.2 What is the overall epidemic prevalence level of animal brucellosis in your country? (Single choice)

☐ No epidemic / Disease-free
☐ Low prevalence (sporadic cases only)
☐ Medium prevalence (regional sporadic outbreaks)
☐ High prevalence (widespread endemic)

2.3 Does your country have confirmed human brucellosis cases caused by livestock infection? (Single choice)

☐ No human cases reported
☐ Sporadic human cases every year

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☐ Regional clustered human cases
☐ Continuous large-scale human infection cases

2.4 What is the reported human brucellosis situation?

☐ High incidence (endemic)
☐ Sporadic cases
☐ Rare/eliminated
☐ No publicly available data

Part 3. Brucellosis Vaccines for Livestock

3.1 Does your country use commercial vaccines for brucellosis prevention and immunization of cattle and sheep? (Single choice)

☐ Yes ☐ No

3.2 If yes, please fill in the detailed information of the brucellosis vaccines used in your country:

Vaccine Specific Name	Source (Domestic/Imported)	Immunized Animals (Cattle/Sheep/Both)

3.3 If no brucellosis vaccine is applied in your country, please briefly state the reason: _____

Part 4. Brucellosis Diagnostic Reagents

Your country's commonly used brucellosis detection reagents include standard detection methods such as RBT (Rose Bengal Test), FPA (Fluorescence Polarization Assay), SAT (Standard Agglutination Test), CFT (Complement Fixation Test), and ELISA (Enzyme-Linked Immunosorbent Assay). Please fill in the actual application information:

4.1 Please confirm the diagnostic reagents used in your country and supplement relevant information:

Detection Method	Whether in Use (Yes/No)	Reagent Specific Name	Source (Domestic/Imported)
RBT (Rose Bengal Test)			
FPA (Fluorescence Polarization Assay)			
SAT (Standard Agglutination Test)			
CFT (Complement Fixation Test)			
ELISA (Enzyme-Linked Immunosorbent Assay)			
Other detection methods			

2

Part 5. Distribution of Veterinary Laboratory Network & Affiliation

5.1 How is the veterinary laboratory system structured in your country?

☐ National – Regional – District (3 tiers)
☐ National – Local (2 tiers)
☐ Mainly private/commercial laboratories
☐ Only national reference laboratory(ies)

5.2 Approximately how many laboratories in your country can perform brucellosis serological testing?

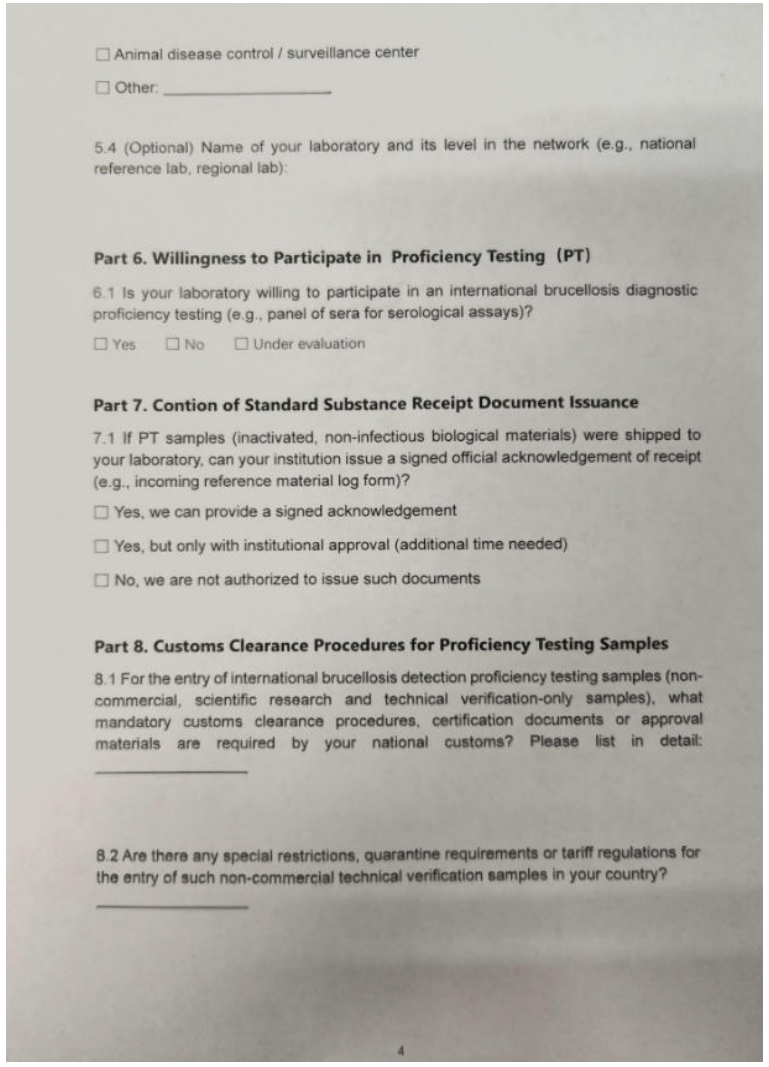
☐ 1–5 ☐ 6–20 ☐ 21–50 ☐ >50 ☐ Uncertain

5.3 Your laboratory / institution is affiliated with:

☐ Ministry of Agriculture / Veterinary Authority (government)
☐ University or public research institute
☐ Private diagnostic company

3

05 Discussion



☐ Animal disease control / surveillance center

☐ Other: _____

5.4 (Optional) Name of your laboratory and its level in the network (e.g., national reference lab, regional lab):

Part 6. Willingness to Participate in Proficiency Testing (PT)

6.1 Is your laboratory willing to participate in an international brucellosis diagnostic proficiency testing (e.g., panel of sera for serological assays)?

☐ Yes ☐ No ☐ Under evaluation

Part 7. Contion of Standard Substance Receipt Document Issuance

7.1 If PT samples (inactivated, non-infectious biological materials) were shipped to your laboratory, can your institution issue a signed official acknowledgement of receipt (e.g., incoming reference material log form)?

☐ Yes, we can provide a signed acknowledgement

☐ Yes, but only with institutional approval (additional time needed)

☐ No, we are not authorized to issue such documents

Part 8. Customs Clearance Procedures for Proficiency Testing Samples

8.1 For the entry of international brucellosis detection proficiency testing samples (non-commercial, scientific research and technical verification-only samples), what mandatory customs clearance procedures, certification documents or approval materials are required by your national customs? Please list in detail:

_____

8.2 Are there any special restrictions, quarantine requirements or tariff regulations for the entry of such non-commercial technical verification samples in your country?

_____

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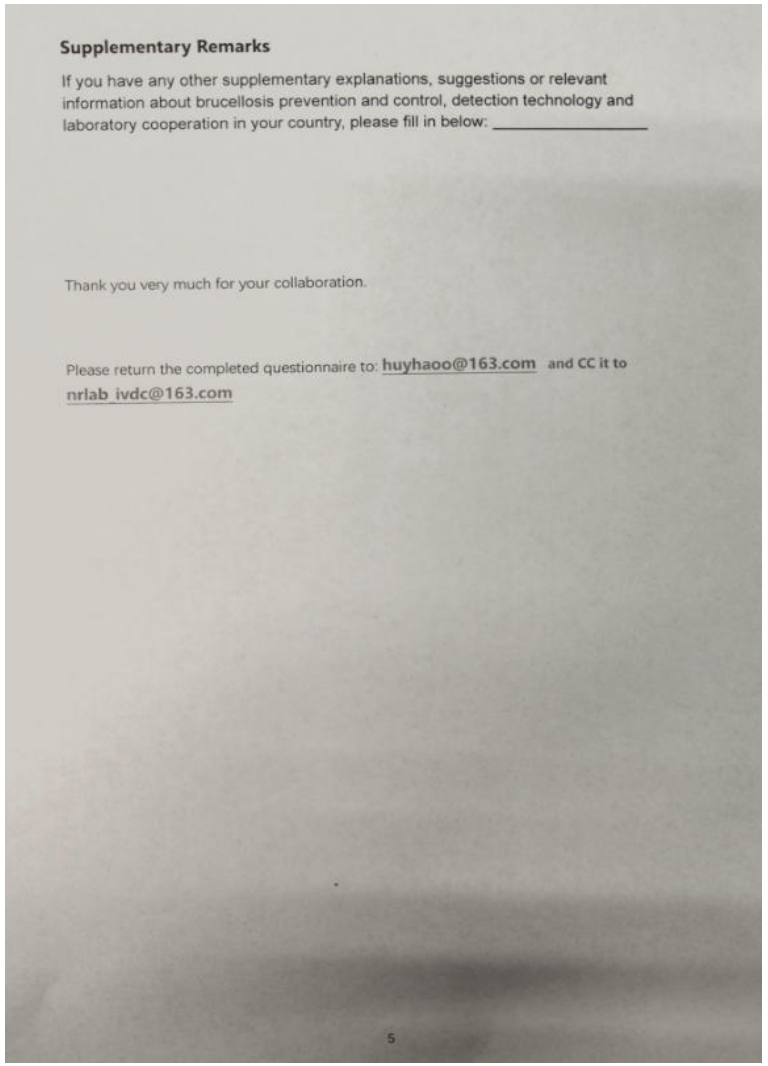

Supplementary Remarks

If you have any other supplementary explanations, suggestions or relevant information about brucellosis prevention and control, detection technology and laboratory cooperation in your country, please fill in below: _____

Thank you very much for your collaboration.

Please return the completed questionnaire to: huyhaoo@163.com and CC it to nrlab_ivdc@163.com

5

Thank you!

Together, advancing brucellosis diagnostic quality across the Asia-Pacific region, in line with WOAH standards.

Enhancing Diagnostic Quality Through Standardisation, Collaboration, and Innovation

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