

[NEPAL]

Nepal's follow-up action on zTB surveillance and control since the 2025 training

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- Nepal records approximately 67,000 new tuberculosis cases and 16,000 deaths each year (National Tuberculosis control center)
- Nepal ranks among the top 30 countries facing a high burden of drug-resistant tuberculosis (WHO)
- TB listed as top 10 prioritized zoonotic disease.
- TB detected in cattle, buffalo, captive elephants and wildlife.
- Cattle and buffalo form the core of Nepal's livestock economy
- Close contact between livestock and rural households, which increases the risk of TB transmission
- Department of Livestock Services in co-ordination with provincial and local level is responsible for Bovine TB diagnosis, surveillance and control.



- **Wildlife sector**

Nepal Elephant TB Control and Management Action Plan (2011–2015) and now included in annual programme of DNPWC

- **Animal Health Sector**

No dedicated act/policy yet

Animal health and livestock service act and regulation

Slaughter house and meat inspection act and regulation

- **Public Health Sector**

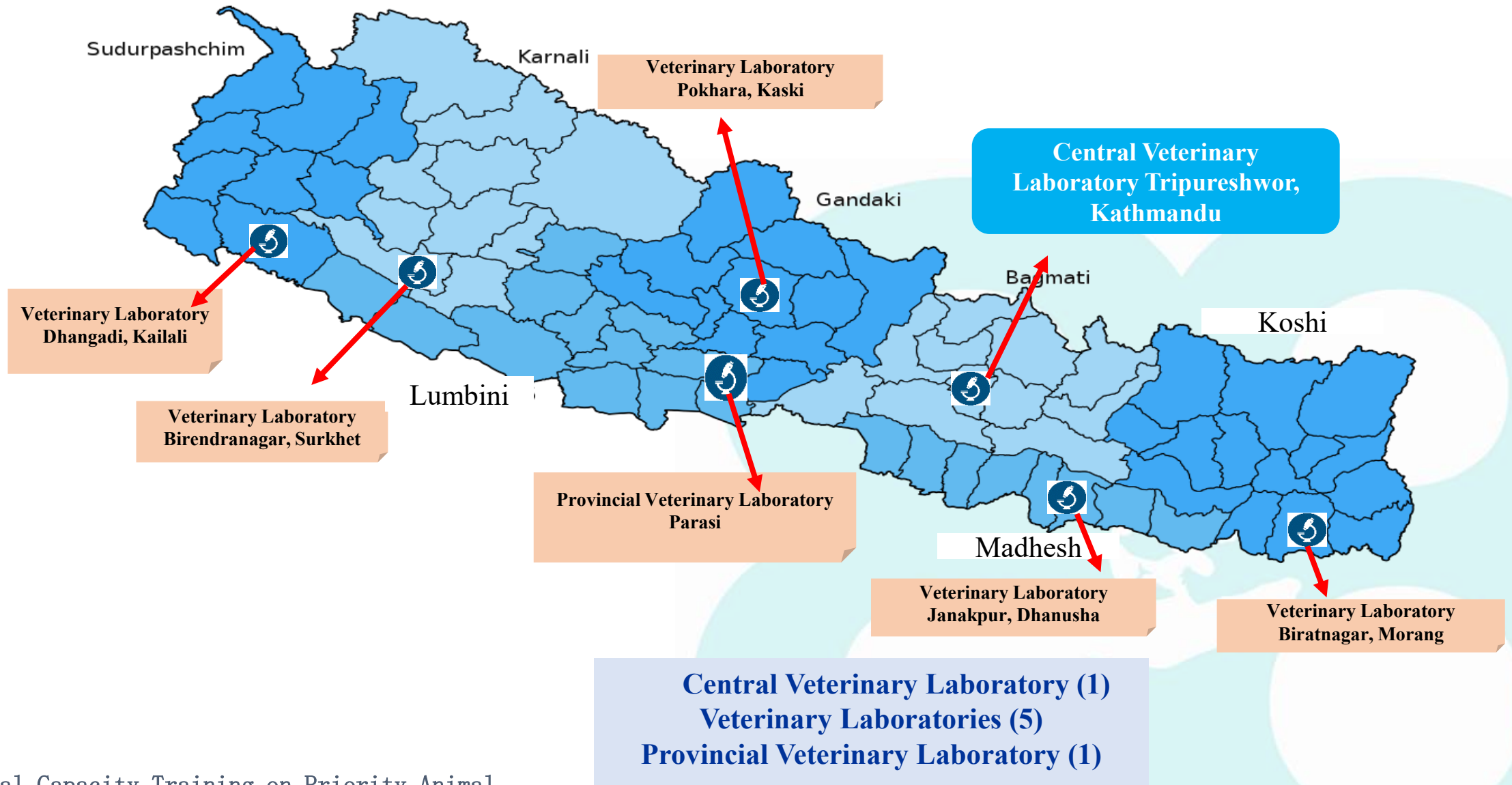
National Strategic plan to end Tuberculosis in Nepal (2021/22- 2025/26)

- ✓ Decrease incidence from 238 in 2020/21 to 81 per 100000 population by 2025/26
- ✓ Decrease mortality from 58 in 2020/21 to 23 per 100000 population by 2025/26



Laboratory Network

4





Diagnostic Facility – Animal Health Sector

5

Intradermal Tuberculin Test

Primary field-level screening method for cattle and buffalo

Lateral Flow Assay

Many False positive results

ELISA (Antibody & γ -IFN)

More frequently used

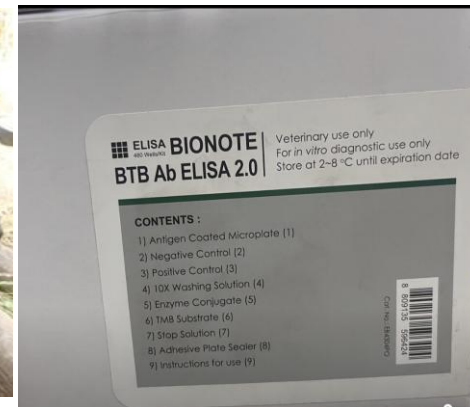


Culture & Isolation

Limited by current biosafety infrastructure

qPCR

Initiated; formal validation with reference laboratory required





Knowledge and Skills Acquired (2025 Training)

6

- ✓ Gained knowledge of WOAHA standards for zoonotic TB /Bovine TB control.
- ✓ Improved understanding of recommended diagnostic tests and their appropriate application in animals.
- ✓ Learned the principles, application, procedures, interpretation, and limitations of
 1. Tuberculin Skin Testing
 2. Lateral flow assay
 3. Bacterial Culture & Staining
 4. Interferon-Gamma Release Assay
 5. ELISA Antibody Testing
 6. PCR
 7. RAA
- ✓ Improved understanding of SOPs, GLP, accreditation, and regulatory compliance.
- ✓ Learned the importance of laboratory PT, quality assurance, and identifying common diagnostic errors.
- ✓ Strengthened the ability to link field screening and laboratory confirmation with surveillance, and animal-health control decisions.

Follow-up Activities After Return

7

- ✓ Technical briefing and internal knowledge-sharing sessions conducted with laboratory staff.
- ✓ Review and refinement of SOPs for TB diagnosis according to WOAH standards.
- ✓ Identification of gaps in routine surveillance and laboratory capacity.
- ✓ Continued inclusion of bovine TB screening within the annual disease investigation program of veterinary laboratories
- ✓ Emphasis was placed on improving specimen collection, labelling, transport, and biosafety to support reliable laboratory diagnosis.
- ✓ Identification of priority districts for expanded cattle and buffalo serological surveillance



Application of acquired knowledge

8

SOPs made for whole blood activation and gamma interferone ELISA procedure

SOP for whole blood activation for gamma interferone TB ELISA (ID vet PPD)

1. Collect blood
 - Collect whole blood aseptically in a heparinized blood collection tube (Do not use EDTA)
2. Mix the blood
 - Immediately after collection, gently invert the tube several times to properly mix the blood with heparin.
3. Transport/store blood
 - Maintain blood at 16–22°C. Preferably process within 8–10 hours. Do not exceed 16 hours after collection.
4. Prepare activation plate
 - Use a sterile 96-well cell-culture plate.
5. Mix blood
 - Gently mix the whole-blood sample several times before dispensing.
6. Aliquot blood
 - Dispense 200 µL whole blood into each activation well. We need four well for one blood.
7. Add stimulation reagents
 - Add 20 µL of the appropriate stimulant to each 200 µL blood aliquot:
 - PBS → negative control
 - PPD avium → non-specific antigen
 - PPD bovis → specific antigen
 - Mitogen → positive control
8. Mix
 - Mix the plate for 1–5 minutes on an agitator (approximately 500 rpm.)
9. Incubate
 - Incubate at 37 ± 2°C in a humidified incubator. Incubation time: 16–24 hours.
10. Centrifuge
 - After incubation, centrifuge the blood for 10 minutes at 500 × g.
11. Collect plasma
 - Carefully collect the plasma supernatant. Avoid aspirating red blood cells, because RBC contamination can interfere with the ELISA result.
12. Test or store plasma
 - Test immediately using the appropriate IFN-γ ELISA, or:
 - 5 ± 3°C: up to 72 hours
 - ≤ -16°C: up to 3 months
 - For longer storage: ≤ -70°C.

SOP – ID Screen® Ruminant IFN-γ ELISA

1. Reagent Preparation

Step	Action
1	Bring all reagents to room temperature (21 °C ± 5 °C)
2	Reconstitute Positive Control with solvent (volume on vial). Mix gently after 5 min.
3	Prepare Wash Solution 1× (dilute 20× concentrate in distilled water)
4	Prepare Conjugate 1× (dilute 10× conjugate 1:10 in Dilution Buffer 1) – just before use

2. Sample Loading (Protocol 2)

Well	Volume
All wells	25 µl Dilution Buffer 1
A1, B1 (Negative Control)	+ 25 µl Negative Control
C1, D1 (Positive Control)	+ 25 µl Positive Control
All other wells (samples)	+ 25 µl sample

3. Procedure

Step	Action	Condition
1	Agitate plate	2 min ± 1 min, RT
2	Incubate	1 h ± 5 min, 37 °C
3	Wash	6 × 300 µl Wash Solution
4	Add Conjugate 1×	100 µl / well
5	Incubate	1 h ± 5 min, 37 °C
6	Wash	6 × 300 µl Wash Solution
7	Add Substrate (TMB)	100 µl / well
8	Incubate	15 min ± 2 min, RT, dark
9	Add Stop Solution	100 µl / well
10	Read OD	450 nm

4. Validation

- Mean OD_PC > 0.500
- OD_PC / OD_NC > 3

5. Interpretation (Protocol 2)

$$S/P\% = (OD_{activated} - OD_{control}) / (OD_{PC} - OD_{NC}) \times 100$$

S/P %	Result
< 35 %	Negative
≥ 35 %	Positive

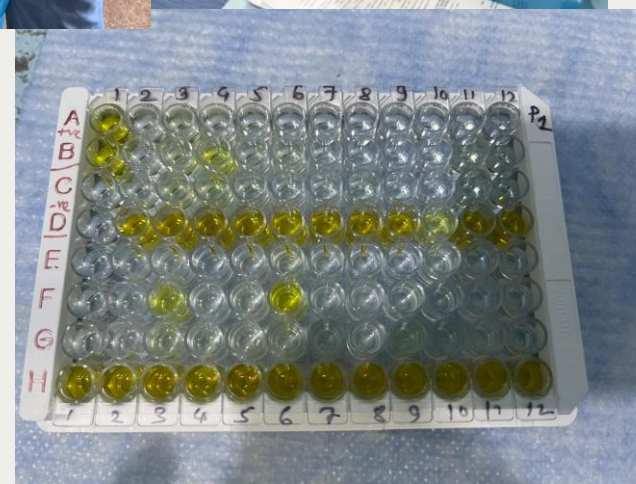
Note: Caution if both PPDavium and PPDbovis OD > 2.5.

Application of acquired knowledge

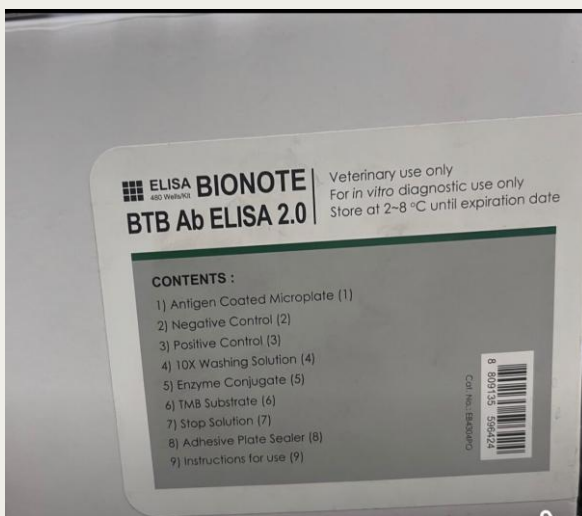
Gamma Interferone ELISA conducted using ID vet ELISA Kit



Total sample tested – 68
Positive sample- 3



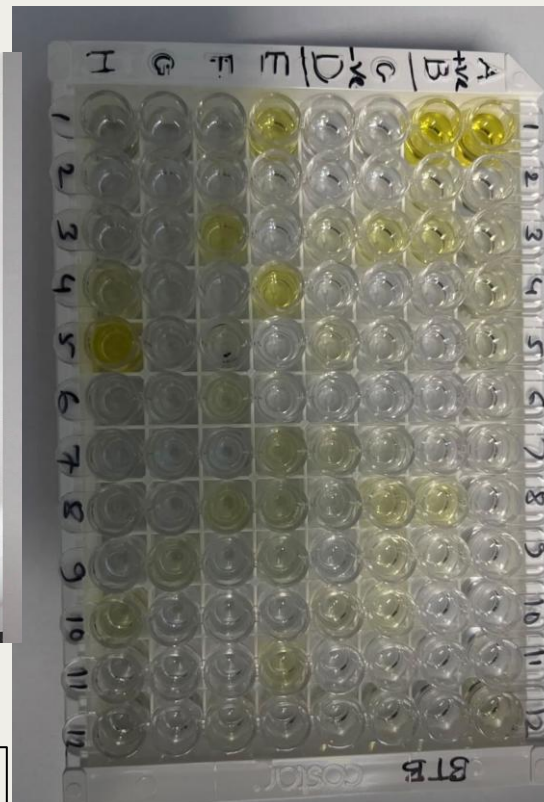
Antibody ELISA conducted using Bionote ELISA Kit



1	2	3	4	5	6	7	8	9	10	11	12
+ve	MG6A	MM10	MG1B	MB6A	MG3A	MG11A	MG5B	MK3	MK11	KA-7	BK-9
+ve	MG7A	MM3	MK13B	MB13	MB5A	MG2A	MG10A	MG3B	MK2A	KA-6	BK-5
-ve	MG14A	MM1	MM9	MG3C	MB12	MG4A	MG2A	MG5A	MG2B	KA-11	BK-6
-ve	MB4B	MM2	MK15	MM8	MM14	MG11A	MG5A	MG6B	MG8A	KA-12	BK-7
EMK8	MG15	MK9	MK7	MM6	MM7	MM9	MB14	MB2A	MB5B	KA-13	BK-8
FMB14	MM11	MM12	MG8B	MK6	MK14	MK10	MM15	MB10	MB1A	KA-14	BK-9
G	MG15A	MB8A	MB7A	MG13A	MG7B	MK5	MK13	MM5	MM3	MB3	BK-1
H	MG15B	MG5A	MK1A	MG4A	MG12A	MG6B	MK4	MK12	MM16	KA9	BK-2

1st Incubation : 1:29pm to 2:29pm
2nd Incubation : 2:42pm to 2:58pm.

Test: Bovine TB Antibody ELISA
Date: 2026 May 19
Conducted by: Dr. Anil & Rikesh.



Total sample tested – 92
Positive sample- 0



Key Challenges

- Absence of dedicated national control strategy for bovine TB
- Cattle slaughter is prohibited in Nepal, so the usual test-and-slaughter strategy is not practical
- Limited specialised diagnostic capacity, reagents and trained personnel at both field and laboratory levels
- Insufficient surveillance coverage
- Weak formal coordination mechanisms between veterinary services and the national human TB programme.
- Resource and logistical constraints that limit expansion of advanced diagnostic methods

Lessons Learned

- A multi-test diagnostic approach increases result confidence
- Stronger One Health coordination between animal and human health sectors is indispensable
- Participation in regional laboratory proficiency testing schemes will accelerate quality improvement



Future priorities and capacity building needs

12

- Develop a specific animal-health policy for bovine TB control in Nepal.
- Development of feasible control options suited to Nepal's legal framework.
- Strengthen routine surveillance and targeted testing in dairy herds and high-risk areas.
- Upgrade diagnostic infrastructure and staff training for bovine TB detection- Expansion to sub-national level
- Increase One Health coordination between veterinary and human health sectors.
- Participation in PT
- Refreshment training on bovine TB diagnosis and surveillance.
- Foster regional collaboration and technical exchange on zTB diagnosis and control.

Thank you!

Contact information

WOAH Regional Capacity Training on Priority Animal
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Qingdao, China