

- Microscopic Examination
- Purification Process
- Bio-safety and Quality Management

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Microscopic Examination



Clinical Signs of Bovine Tuberculosis

- ◆ Fever, anorexia, progressive emaciation, caseous necrotic nodules
- ◆ **Pulmonary TB**: early fatigue, short dry cough progressing to purulent moist cough; in severe cases dyspnea and a marked drop in milk yield
- ◆ **Udder TB**: enlargement of the supramammary lymph nodes, decreasing milk yield, and cessation of lactation in severe cases
- ◆ **Intestinal TB**: mainly in calves; rapid emaciation, abdominal pain and intractable diarrhea, with feces mixed with mucus and pus
- ◆ **Genital TB**: increased libido, repeated estrus without conception, and abortion
- ◆ **Cerebral TB**: neurological signs and blindness





Pathological Symptoms of Bovine Tuberculosis

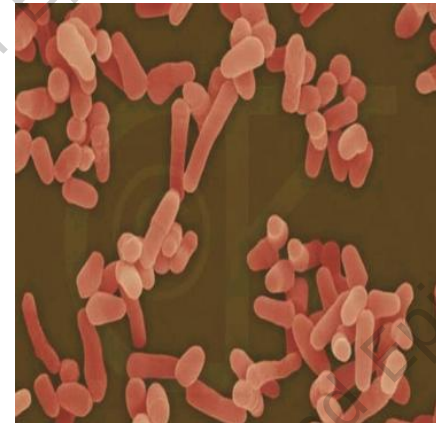
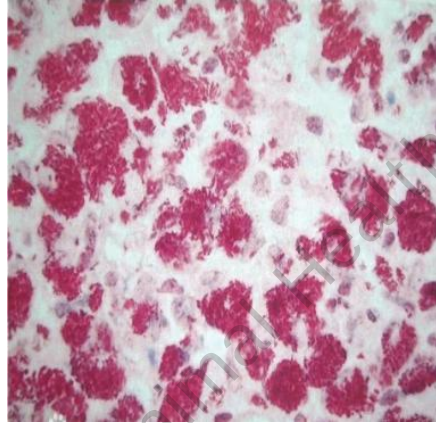


- A: Tuberculous cavitation in the liver
- B: Nodules in the mammary lymph nodes
- C: Nodules in the gastric lymph nodes
- D: Nodules in the mesenteric lymph nodes
- E: Nodules in the spleen
- F: Nodules in the intestinal lymph nodes
- G: Nodules in the hilar lymph nodes
- H: Nodules in the lungs
- I: Miliary multiple tuberculosis of the thoracic pleura ("pearl-like")



Morphology of Mycobacterium bovis

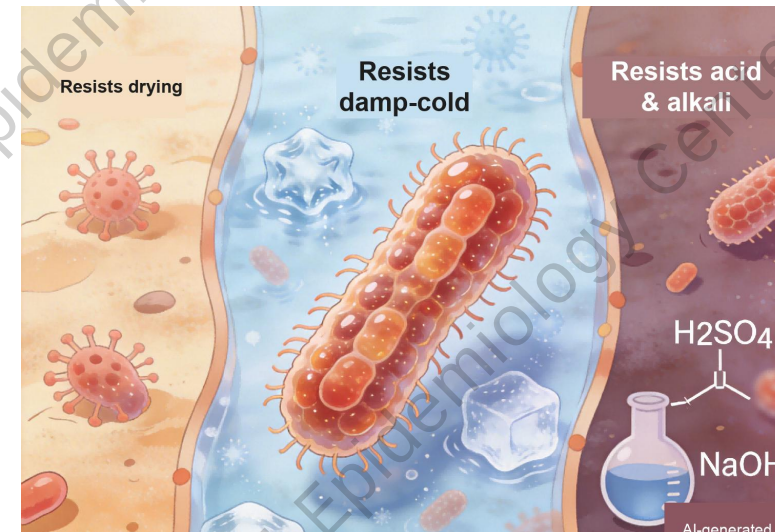
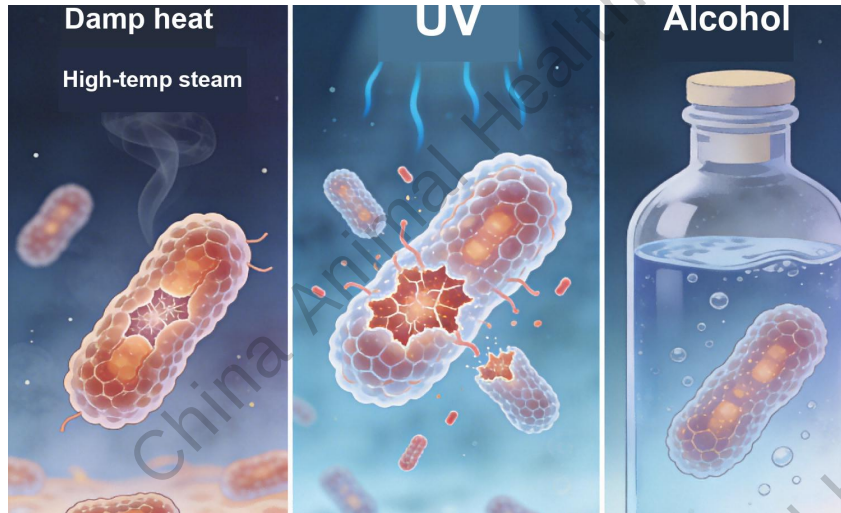
- ◆ **M. bovis cells are short and thick, stained red by acid-fast staining**
- ◆ **Colonies are rough, opaque, granular, creamy white or pale yellow**





Resistance of *Mycobacterium bovis*

“Three Fears”: moist heat, UV light, alcohol



“Three Not-Fears”: dry, damp-cold, acid/alkali



Bacteriological Culture

Aseptically collect organs:
lung, lymph nodes, liver, etc.



ZN acid-fast staining + culture
+ molecular identification

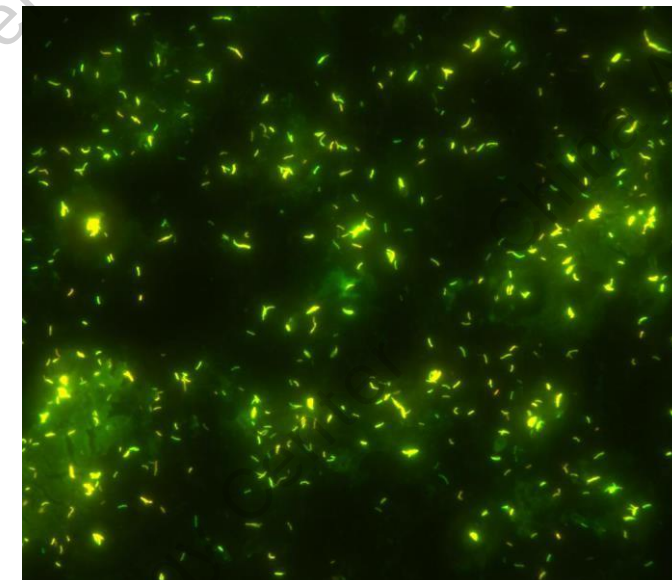
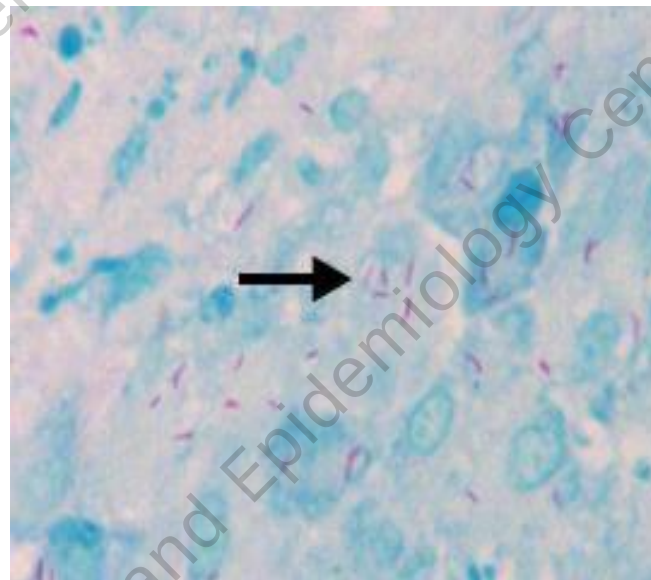


- ◆ Gold standard for laboratory diagnosis of TB: culture of *Mycobacterium tuberculosis*
- ◆ *M. tuberculosis* has high nutritional requirements and requires specific culture media
- ◆ Requires a biosafety level 3 (BSL-3) laboratory
- ◆ Colonies: rough, raised, granular, yellow or white



Bacteriological Detection

- ◆ Most countries use histopathology and bacteriology in slaughterhouse surveillance
- ◆ **Smear-stain microscopy detects mycobacteria:**
the specimen is smeared and stained
- ◆ There are 2 types of mycobacterial smear microscopy

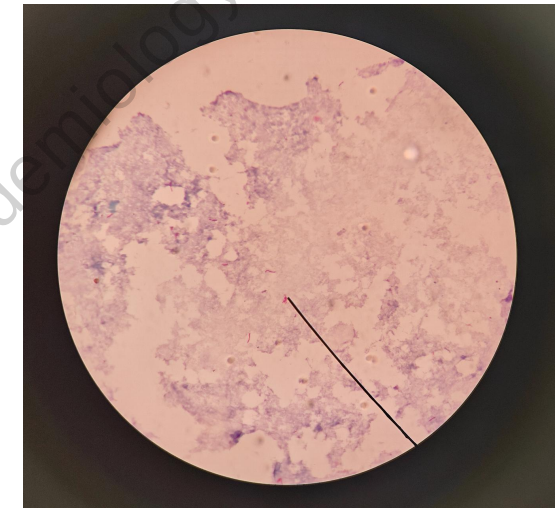
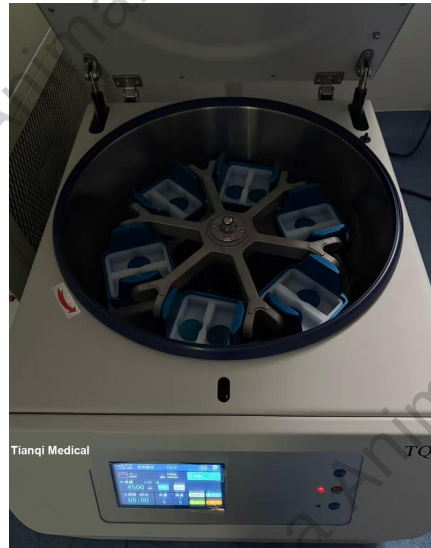
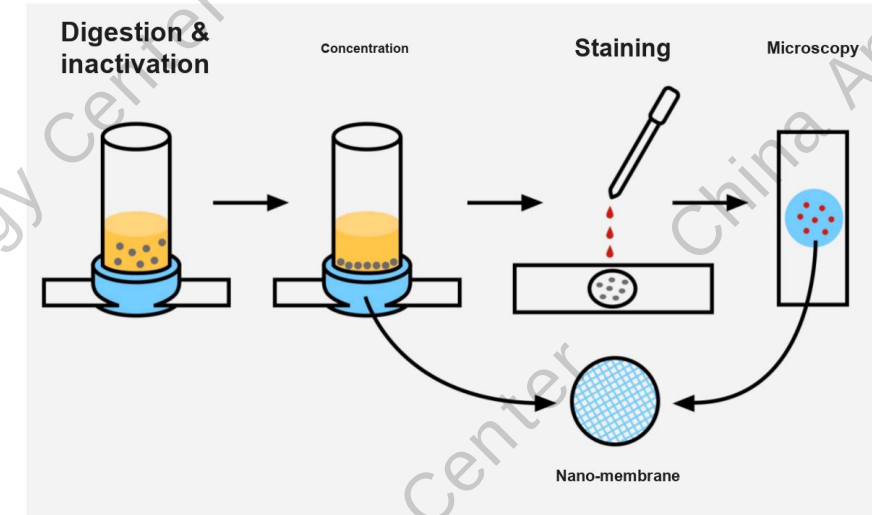


Method	Advantages	Disadvantages
Ziehl-Neelsen acid-fast staining	Simple, economical, specific, fast	Low sensitivity
Fluorescent staining	Simple operation, rapid detection, high positivity (few bacilli)	Darkroom operation; high maintenance cost



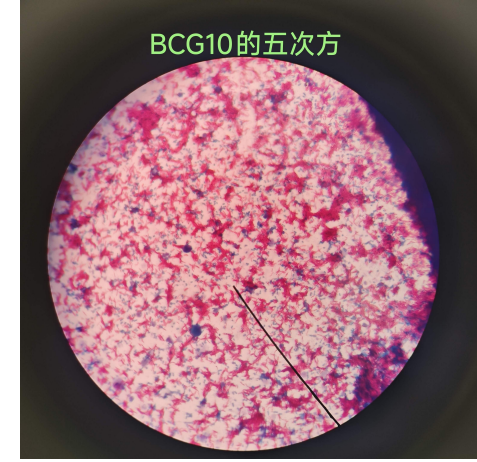
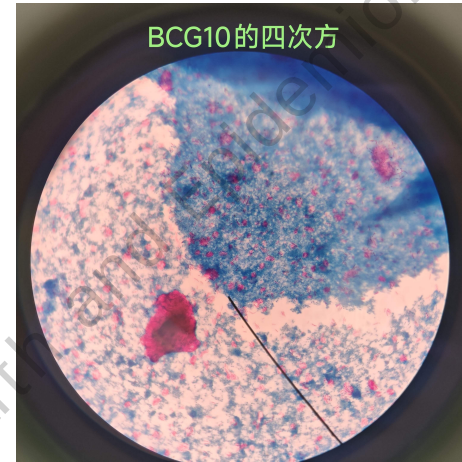
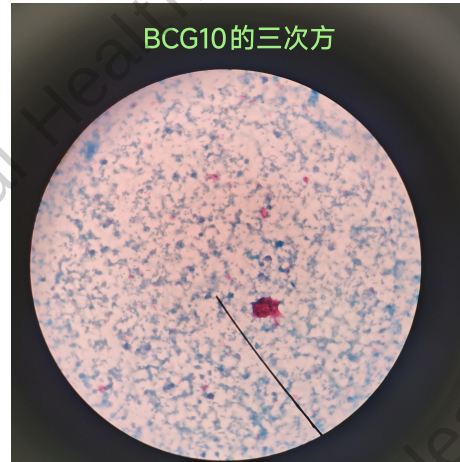
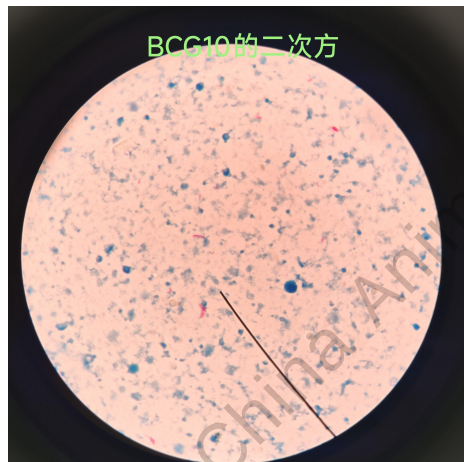
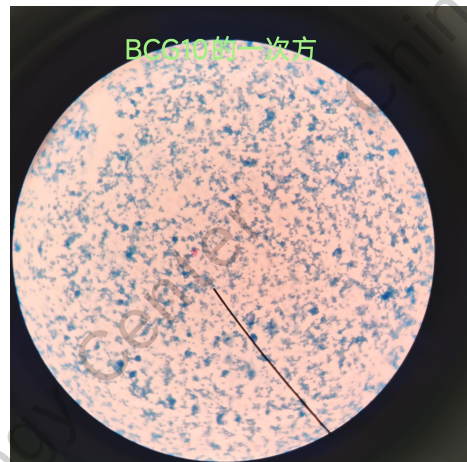
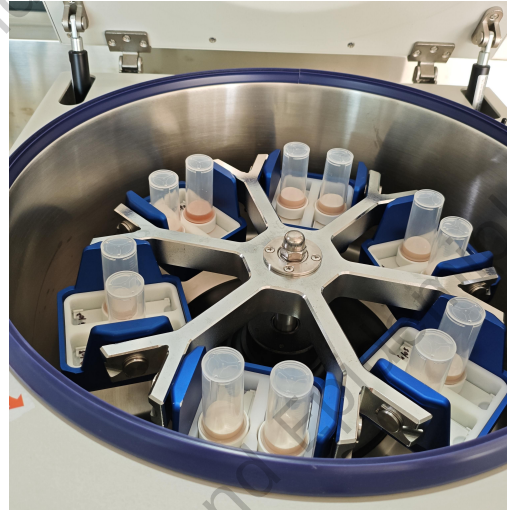
Enrichment + Microscopy

A special polymer microporous membrane
captures mycobacteria; after enrichment, the
sample is stained and examined by microscopy





Enrichment + Microscopy





Enrichment + qPCR

Detection after enrichment

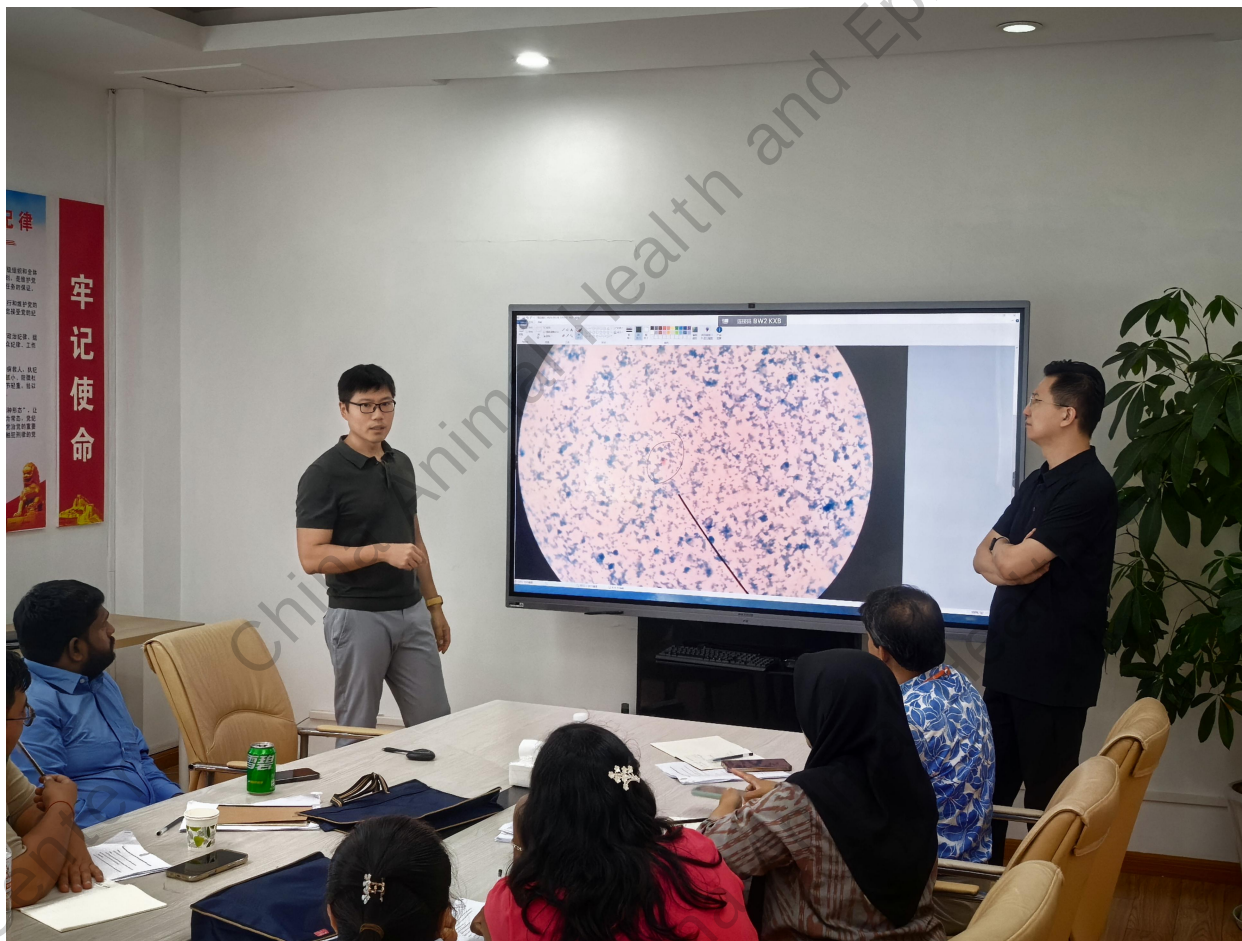
Simulated Milk Samples

Directly extract nucleic acids

V.S

Extract nucleic acids after enrichment

	IS6110	MPB70	IS1081
Direct	31.578	31.878	29.707
Enriched	29.454	29.348	27.262





II

Purification Process

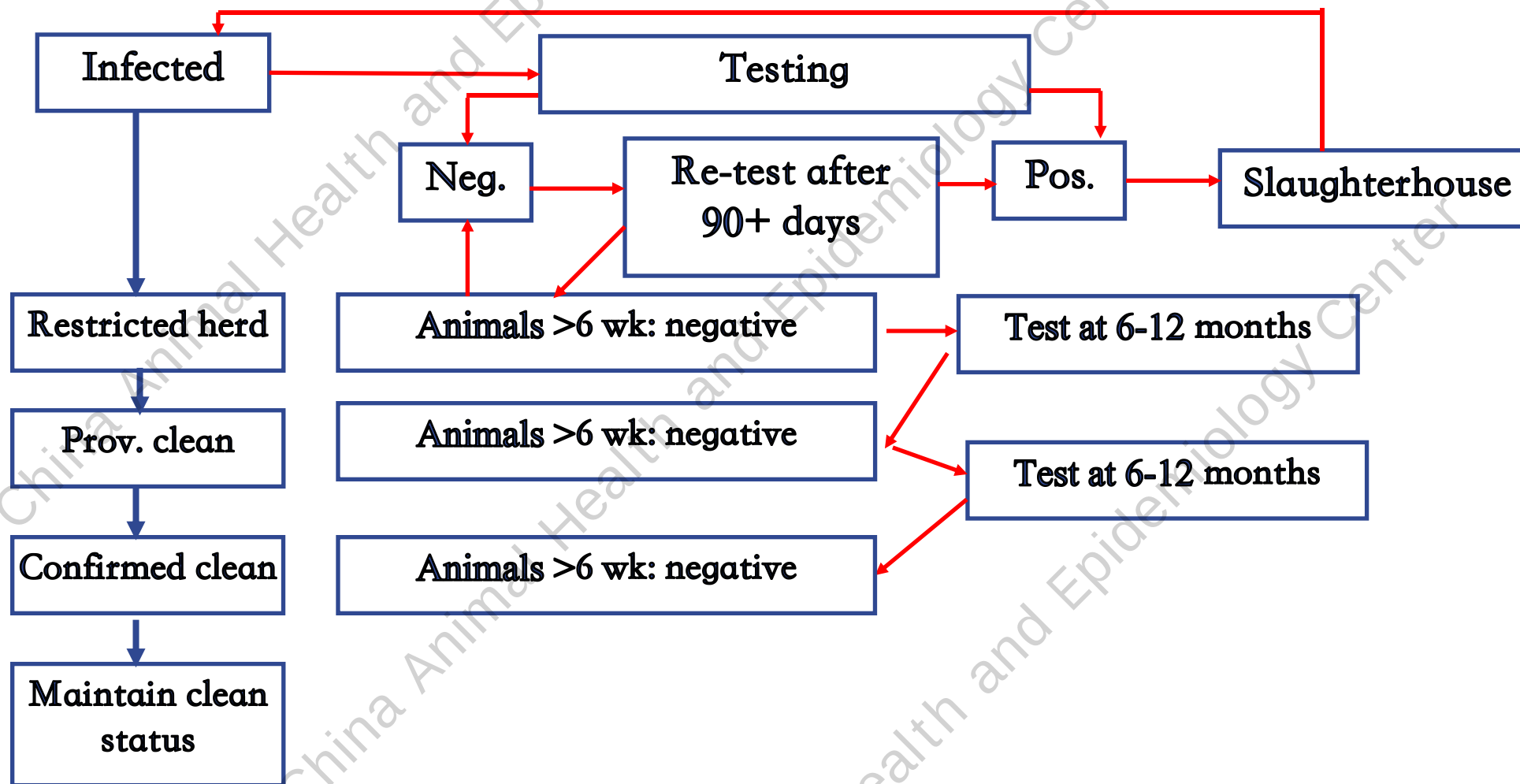


Eradication Standards for Bovine Tuberculosis

- ◆ Animal MTBC infection is a nationally notifiable disease;
- ◆ At least in the past **12 months**, no MTBC infection;
- ◆ At least in the past **12 months**, no animal in the herd has shown clinical signs or lesions of MTBC infection in ante-mortem or post-mortem examinations;
- ◆ The first test of animals over 6 months after the last case is culled is performed on animals over 6 weeks of age; the second test is performed at least **6 months later; both tests must be negative**;
- ◆ Introduced animals and their germplasm comply with relevant disease-free requirements;
- ◆ At least in the past 12 months, no other herd on the same premises has had MTBC infection;
- ◆ Or, measures have been taken to prevent spread of infection to other herds.



Eradication Procedure for Bovine Tuberculosis





III

Bio-safety and Quality Management



Proper Use of Masks

Prevents infectious material splashing onto oral/nasal mucosa.





Glove Selection and Double Gloving



First layer: nitrile gloves

Good mechanical strength, abrasion resistance, puncture resistance and chemical resistance

Second layer: latex gloves

Excellent elasticity with certain abrasion, tear and cut resistance



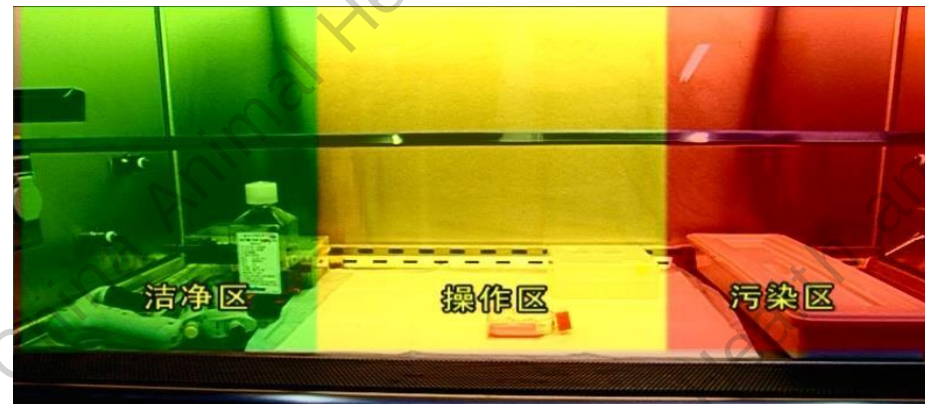


Biological Safety Cabinet

Provides triple protection for the operator, experimental samples and the external environment through directional airflow and a HEPA filtration system

Preparations before use

- Raise the front window to the specified height and preheat for 3 min.
- Lay bench paper, attach waste bags to the side walls, and prepare sharps containers and disinfectant.
- Place experimental materials in zones inside the cabinet; keep only the items needed for the current test.



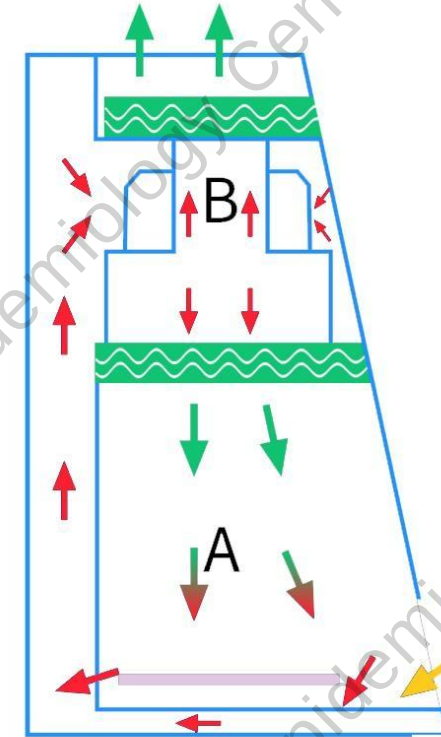


Biological Safety Cabinet

Precautions

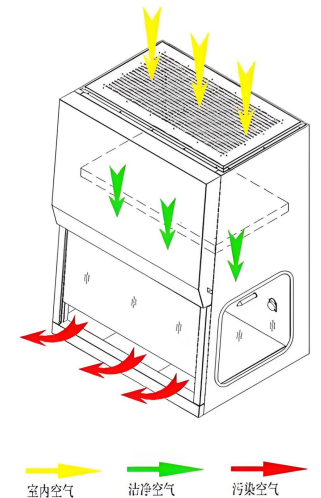
- **Always distinguish from a laminar flow hood (clean bench)**
- Do not use an alcohol lamp;
- Do not block the intake grille with paper, equipment or other items;
- Place aerosol-generating equipment (e.g., vortex mixers, centrifuges) toward the rear of the cabinet;
- To prevent splashed droplets from contaminating the work surface, perform the experiment on bench paper.

Type A2 BSC

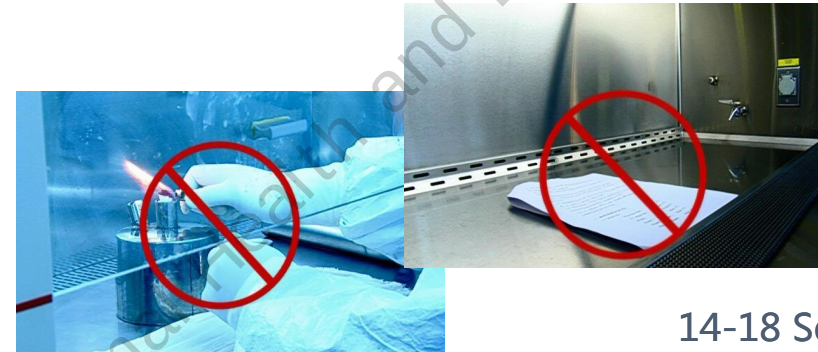


Room air
Polluted air
Purified air
A: Protected
B: Contam.

Clean bench



室内空气 洁净空气 污染空气

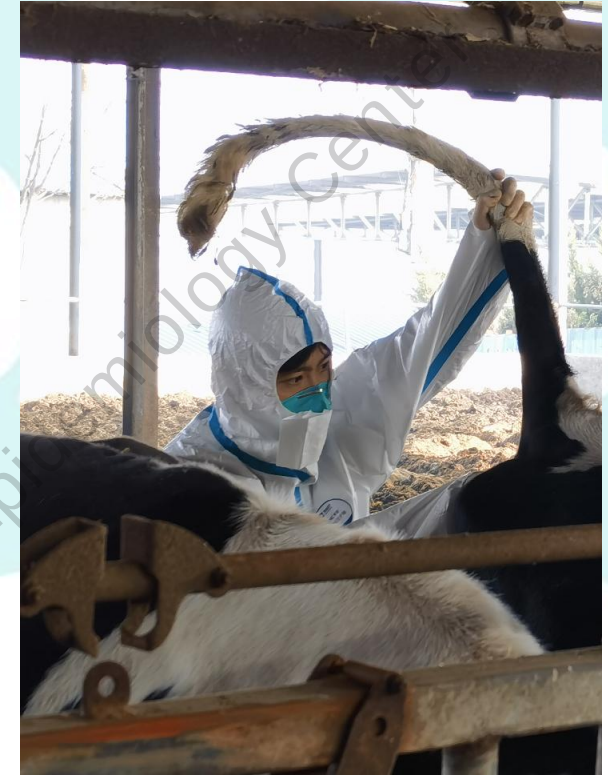




Field Investigation

Field investigation: the most critical and complex step.

Assess risks, Identify causes, Discover target, Take protective measures





Quality Control

Qualified Samples

01.

- Scientific sampling: representative
- Proper storage: no degradation, no hemolysis
- Clear info: time, place, species, purpose, contact
- Clean, intact packaging

Clear Workflow

Fully traceable, no break

- Handover: sampler → courier → receiver → tester
- Status: proper storage, clear info

02.

Rigorous Methods

03.

- Based on national/industry standards
- SOP implemented
- Instrument calibration and maintenance
- Qualified, well-trained personnel

Comprehensive Controls

- Positive ctrl: verifies detection
- Negative ctrl: no contamination
- Blank ctrl: monitors reagent/water
- Internal ctrl: monitors extraction

04.

