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

Presentation Title: Culture and Crystal Violet Staining of *Brucella*

Country-China

Name of presenter-Dr. Haoran Zhao

2nd WOAH Regional Training Workshop on Brucellosis Diagnosis

Beijing, P.R. China, 26 – 29 May 2026

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

»»» 01 «««

Culture of *Brucella*

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

Brucella culture is categorized into solid culture and liquid culture.

Solid culture

Culture Media

- **Selective Brucella Agar**

  （First choice for isolation of clinical samples）
- **Brucella Agar**

  （Pure culture & colony identification）
- **Blood Agar**

  （For primary isolation）

Purpose & Application

- **Isolate bacteria from clinical samples (placenta, aborted fetus, milk.)**
- **Purify strains and observe typical colony**
- **Strain preservation**
- **Single colony picking**

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

Liquid culture

Culture Media

- **Brucella Broth**
- **Tryptic Soy Broth (TSB)**

Purpose & Application

- **Bacterial enrichment and large-scale cultivation**
- **Strain subculture and preparation of bacterial suspension**
- **Dynamic observation of bacterial growth**

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

General Incubation Conditions

- **Temperature： 37 ℃ (optimal temperature)**
- **Gas environment： 5% CO_2 (adapts to all Brucella biovars)**
- **Incubation time：**

▶ Solid culture: 3 ~ 10 days (slow-growing bacteria, do not discard plates early)

▶ Liquid culture: 2 ~ 5 days

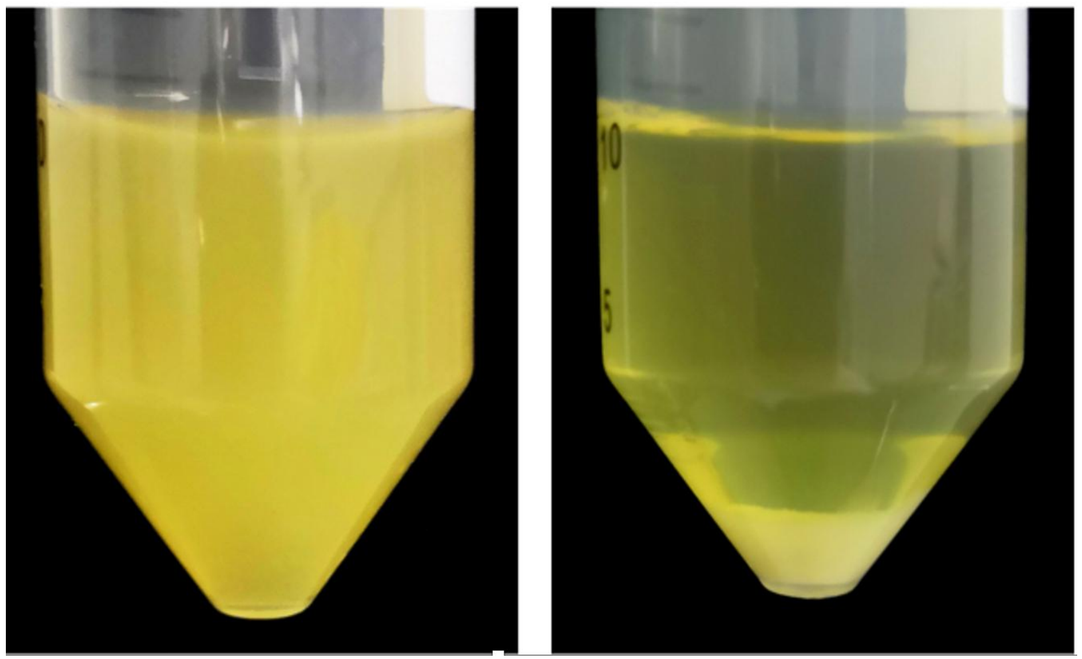
World Organisation for Animal Health

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

Solid culture

Brucella Culture on Tryptic Soy Agar (TSA)

Liquid culture



Brucella Heat Agglutination Test:
Smooth *Brucella* (Left) , Rough *Brucella* (Right)

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

»»» 02 «««

Crystal Violet Staining of *Brucella*

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

Experiment objective

Differentiation between Smooth and Rough *Brucella* (S/R Typing)

Applications

- Preliminary virulence assessment of clinical isolates
- Stability monitoring of laboratory passaged strains
- Purity detection of vaccine strains

Principle

- Smooth Brucella strains possess intact smooth lipopolysaccharide (S-LPS) with O-antigen side chains on their surface, which blocks the penetration of crystal violet, so the colonies remain unstained.
- In rough Brucella strains, S-LPS is deficient or structurally abnormal with loss of O-antigen side chains. This increases the permeability of the cell wall, making the bacteria readily stained by crystal violet.

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

Protocol

01 Sample Preparation

1. **Streak test strains or conduct 10-fold serial dilution.**
2. **Inoculate appropriate dilutions onto TSA.**
3. **Incubate at 37 ° C for 72–96 h (colony diameter: 0.5–1 mm).**
4. **Inoculate standard S & R strains as parallel controls.**

02 Stain Preparation

1. **Solution A: Dissolve 2.0 g crystal violet in 20 mL anhydrous ethanol.**
2. **Solution B: Dissolve 0.8 g ammonium oxalate in 80 mL purified water or distilled water.**
3. **Mix Solution A and Solution B to obtain the stock solution. Before use, dilute the stock solution 40-fold with purified water or distilled water.**

03 Staining Procedure

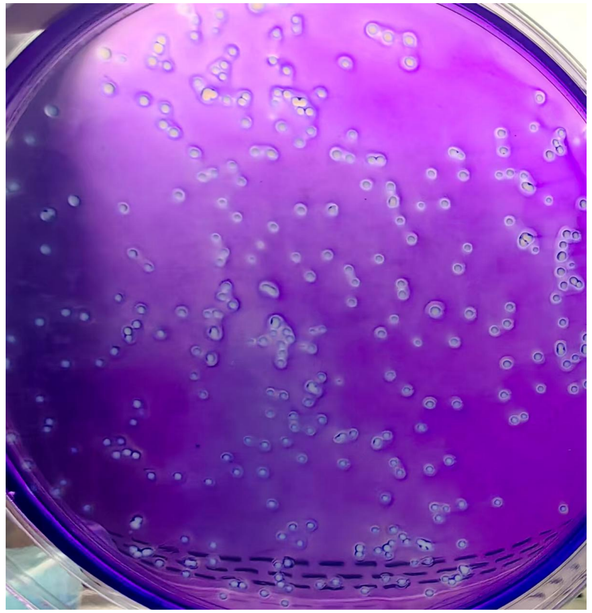
1. **Take agar plates with mature Brucella colonies.**
2. **Cover all colonies with diluted staining solution.**
3. **Stain for 15–20 s.**
4. **Discard the stain and observe colonies immediately with a magnifying glass or microscope.**

World Organisation for Animal Health

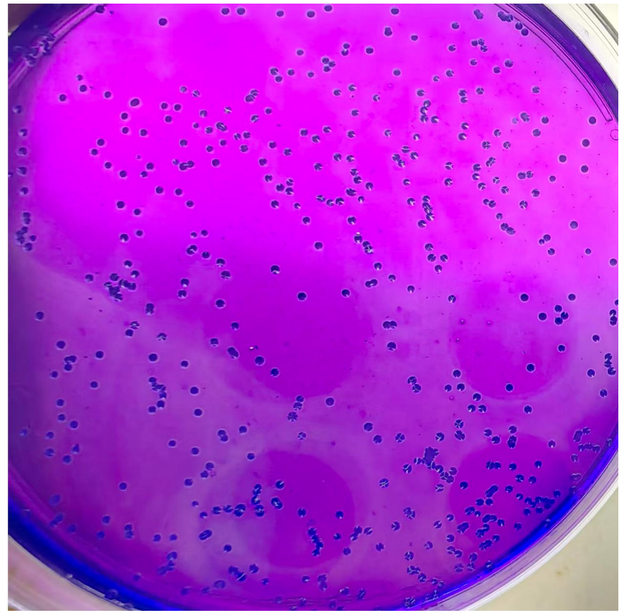
中国兽医药品监察所
China Institute of Veterinary Drug Control
国家动物布鲁氏菌病参考实验室
National Reference Laboratory for Animal Brucellosis

Interpretation of Results

Type	Staining	Colony Morphology
S	Negative (colorless)	Regular, smooth, moist
R	Positive (purple)	Irregular, rough, dry



Smooth (S) colonies: **Unstained; regular & round margins.**



Rough (R) colonies: **Stained purple; irregular & rough margins; occasional cracks.**

Thank you!

WOAH Regional Representation for Asia and the Pacific
Food Science Building 5F - The University of Tokyo
1-1-1 Yayoi, Bunkyo-ku
Tokyo, 113-8657
JAPAN

rr.asia-pacific@woah.org
rr-asia.woah.org

Facebook
X
Instagram
LinkedIn
YouTube
Flickr