

Presentation Title: *Introduction to Brucellosis Diagnostic Methods: Bacterial Culture and Staining Techniques*

布鲁氏菌病诊断方法简介 —— 细菌培养与染色技术
Country: China / 国家: 中国

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Diagnosis

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01

*The staining
characteristics of
Brucella.*

布鲁氏菌的染色特征

02

*The basic procedures
for bacterial culture
and identification.*

细菌培养与鉴定的基本流程

03

*Biochemical and
serological
identification methods.*

关键生化与血清学鉴定方法

Overview of Brucella / 布鲁氏菌概述

Brucella is a genus of gram-negative, nonencapsulated, flagellated, facultatively intracellular coccobacilli.

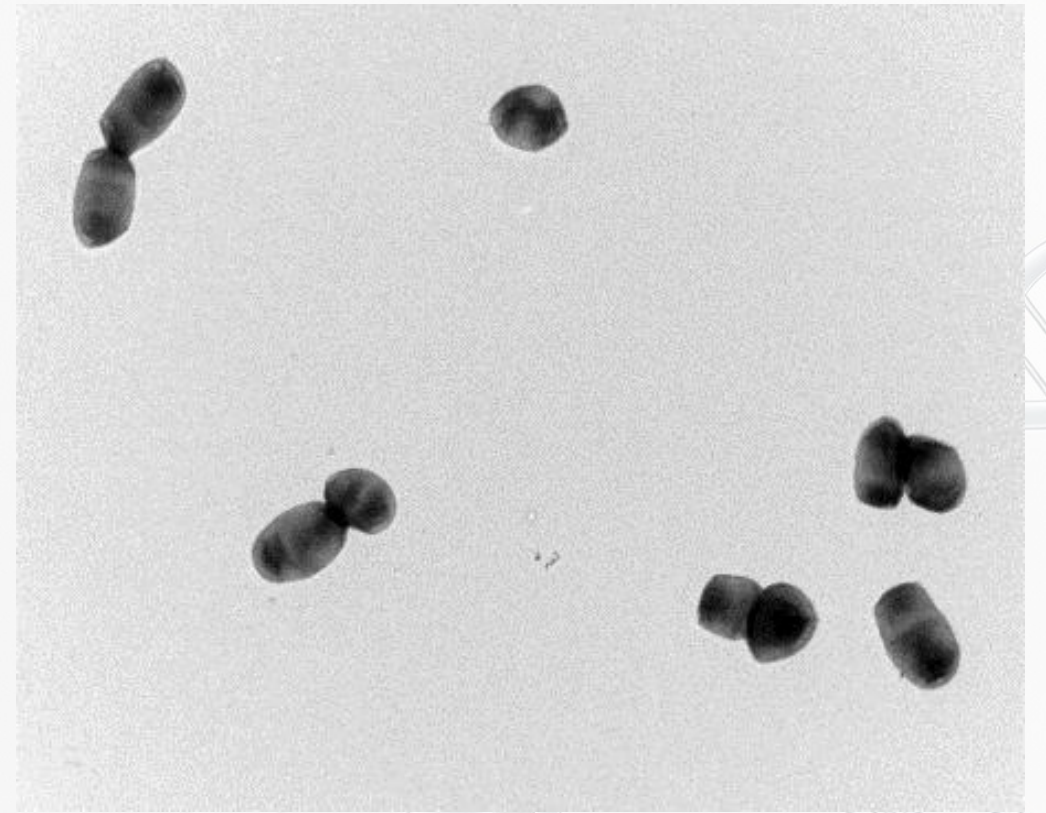
布鲁氏菌是一种革兰氏阴性、无荚膜、具鞭毛、兼性胞内寄生的短杆菌。

Typical size: 0.5-0.7 μm $\times$ 0.6-1.5 μm .

典型大小：0.5-0.7 μm $\times$ 0.6-1.5 μm 。

Primarily causes brucellosis, a zoonotic disease.

主要引起布鲁氏菌病（人畜共患病）。



Modified Ziehl-Neelsen Acid-Fast Staining / 改良萆-尼氏抗酸染色法

Applicable to samples such as whole blood, milk, tissue, Swab and liquid samples.

适用于全血、乳样、组织、拭子和液体等样品。

Staining procedure: Fixation → Phenol fuchsin → Decolorization → Counterstaining.

染色流程：固定 → 苯酚复红染色 → 脱色 → 复染。

Result: Blue background with red bacterial cells.

结果：背景蓝色，菌体红色。

Overview of Bacterial Culture Method / 细菌培养法概述

Tissue samples: For aseptically collected tissues, remove excess portions (e.g., fat), then take 5 g of tissue, cut into small pieces, add 10mL of sterile PBS buffer, and grind. After grinding, take 200 μ L and inoculate onto the surface of the selective medium.

组织样本: 无菌采集的组织, 剔除多余部分 (如脂肪), 取5g组织剪成小块, 加入10mL无菌PBS缓冲液进行研磨后, 取200 μ L接种于选择培养基表面。

Vaginal lavage fluid, semen, synovial fluid, gastric contents, etc.: Take 200 μ L and inoculate onto the surface of the selective medium.

阴道冲洗液、精液、关节液、胃内容物等: 取200 μ L接种于选择培养基表面。

Milk samples: Take 15mL, centrifuge at 2000r/min for 15min, then use a 10 μ L inoculating loop to take 2 loops from the cream layer and inoculate onto the surface of the selective medium.

乳样: 取15mL, 2000r/min离心15 min, 用10 μ L接种环取奶油层2环, 接种于选择培养基表面。

Overview of Bacterial Culture Method / 细菌培养法概述

Each sample spread onto two plates.

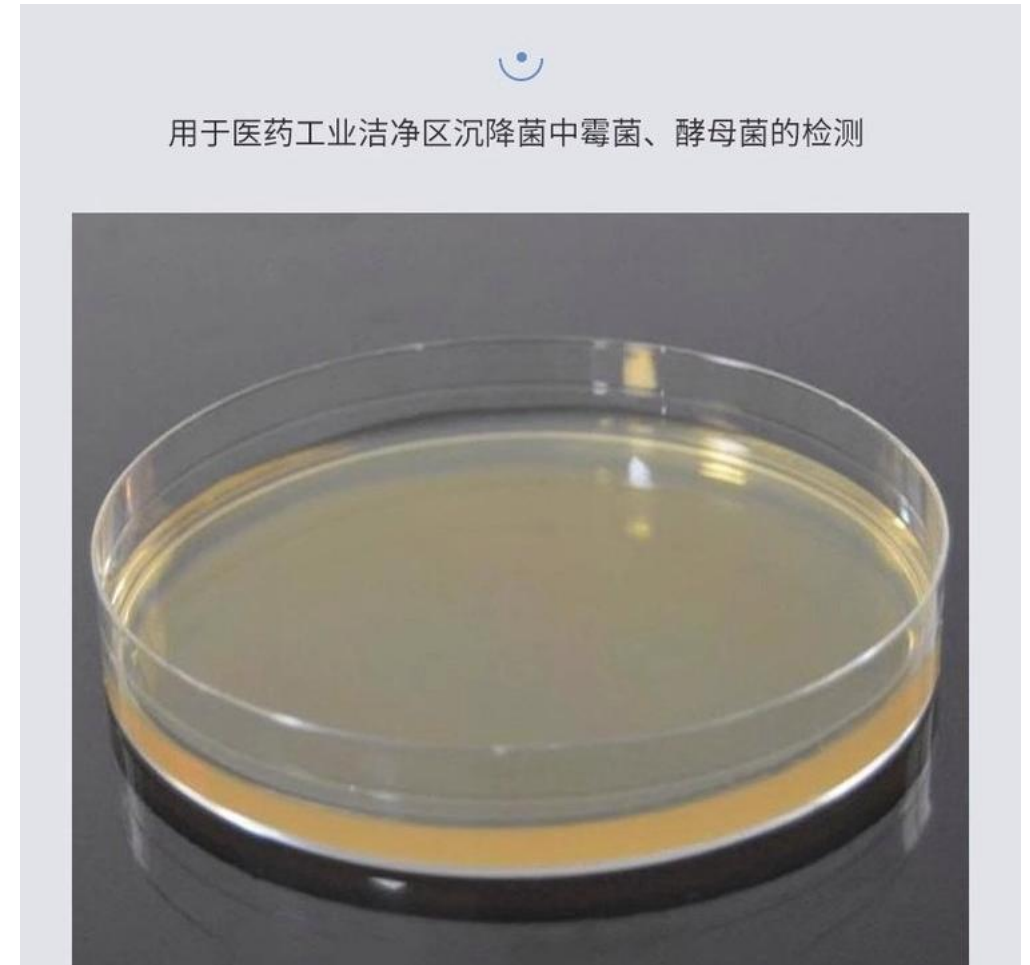
每份样品涂布两个平板。

Incubate in both regular and 5% to 10% CO₂ incubators.

置于普通培养箱和含5%至10% CO₂ 的培养箱中。

After sample spreading, incubate at 37°C for 3-10 days.

样品涂布后，37°C培养3-10天。



Colony Morphology and Characteristics / 菌落形态与特征



Round shape, 1-2 mm in diameter.

圆形，直径1-2 mm。

Smooth edge, off-white color, slightly raised.

边缘光滑，灰白色，微隆起。

Darken and enlarge over time.

随时间变暗、变大。

Biochemical Identification / 生化鉴定



CO₂ requirement test (Some strains require 5-10% CO₂).

CO₂ 需求试验 (部分菌株需 5-10% CO₂)。

Growth Condition / 生长条件

H₂ S production test (Lead acetate paper strip turns black).

产H₂ S试验 (乙酸铅试纸条变黑)。

Metabolic Characteristics / 代谢特征

Biochemical Identification / 生化鉴定

Oxidase test (Positive: Bright red color).

氧化酶试验 (阳性: 鲜红色)。

Urease test (Positive: Yellow to pink/purple color change).

脲酶试验 (阳性: 黄色变粉红/紫色)。



Biochemical Identification / 生化鉴定

-----Dye Sensitivity Test / 染料敏感性试验

*Sensitivity to thionine
and fuchsin dyes.*

对硫堇、复红染料敏感性。

*Concentration:
20 $\mu\text{g/mL}$.*

浓度：20 $\mu\text{g/mL}$ 。

*Observe growth after
3-4 days of incubation.*

培养3-4天后观察生长情况。

Biochemical Identification / 生化鉴定

-----Serum Agglutination Test / 血清凝集试验

Use A, M, and R monovalent sera.

使用A、M、R单价血清。

Smooth strains agglutinate with A or M serum.

光滑型与A或M血清凝集。

Rough strains agglutinate with R serum.

粗糙型与R血清凝集。



Biochemical Identification / 生化鉴定

-----Phage Lysis Test / 噬菌体溶解试验

Spread bacterial suspension on agar plates.

菌液涂布于琼脂平皿。

Add standard phage solution dropwise.

滴加标准噬菌体液。

Results: No growth (+), partial growth ($\pm$), good growth (-).

结果：不生长 (+)、部分生长 ($\pm$)、良好生长 (-)。



Identification Table of Brucella Strains

Brucell species	Colony morphology	Serum requirement ^t	Phage lysis					Oxidase	Urease	Preferred host
			Tb		Wb	Iz ₁	R/C			
			RTD ^c	10 ⁴ RTD	RTD	RTD	RTD			
<i>B. abortus</i>	S	— ^d	+	+	+	+	—	(+) ^e	(+) ^f	Cattle and other bovids
<i>B. melitensis</i>	S		—	—	(—) ^g	+	—	+	+ ^h	Goats and sheep
<i>B. suis</i>	S	—	—	+	(+) ⁱ	(+) ⁱ	—	+	+ ^j	Pigs, hares, reindeer, rodents
<i>B. canis</i>	R	—	—	—	—	—	+	+	+ ^j	Dogs
<i>B. Ovis</i>	R	+	—	—	—	—	+	—	—	Sheep

Note: (+)/(-) mostly indicate positive/negative.

a. Phages: Tbilisi (Tb), Weybridge (Wb), Izatnagar (Iz), and R/C.

b. Normal colony morphology: S: smooth, R: rough.

c. RTD: routine test dilution.

d. *Brucella abortus* biotype 2 requires serum for primary isolation.

e. Some African isolates of *Brucella abortus* biotype 3 are negative.

f. Moderate speed; strain 544 and some field isolates are negative.

g. Some isolates are lysed by phage Wb.

h. Slow speed; some isolates are rapid.

i. Some isolates of *Brucella suis* biotype 2 are not lysed or are partially lysed by phage Wb or Iz.

j. Rapid.

Identification Table of *Brucella* Biotypes (1)

<u>Brucell</u> species	Biotype	CO ₂ requirement	H ₂ S production	<u>Dyestuffs</u> ^a		<i>Agglutination</i>			Reference strain
				<u>thionin</u>	fuchsin	A	M	R	
<i>B. abortus</i>	1	(+) ^b	+	—	+	+	—	—	544
	2	(+) ^b	+	—	—	+	—	—	86/8/59
	3	(+) ^b	+	+	+	+	—	—	Tulya
	4	(+) ^b	+	—	(+)	—	+	—	292
	5	—	—	+	+	—	+	—	B3196
	6	—	(—)	+	+	+	—	—	870

Note: (+)/(-) mostly indicate positive/negative.

a. Mass concentration of the dye in serum-glucose medium: 20 µg/mL.

b. Primary isolation is usually positive.

Identification Table of *Brucella* Biotypes (2)

<u>Brucell species</u>	Biotype	CO ₂ requirement	H ₂ S production	<u>Dyestuffs^a</u>		Agglutination			Reference strain
				<u>thionin</u>	fuchsin	A	M	R	
<i>B. abortus</i>	9	(+ / -)	+	+	+	—	+	—	C68
<i>B. melitensis</i>	1	—	—	+	+	—	+	—	16M
	2	—	—	+	+	+	—	—	63/9
	3	—	—	+	+	+	+	—	Ether
<i>B. suis</i>	1	—	+	+	(—)	+	—	—	1 330
	2	—	—	+	—	+	—	—	Thomsen
	3	—	—	+	+	+	—	—	686
	4	—	—	+	(—)	+	+	—	40
	5	—	—	+	—	—	+	—	513
<i>B. canis</i>	无	—	—	+	(—)	—	—	+	RM6/66
<i>B. Ovis</i>	无	+	—	+	(—)	—	—	+	63/290

Note: (+)/(-) mostly indicate positive/negative.

a. Mass concentration of the dye in serum-glucose medium: 20 µg/mL.

b. Primary isolation is usually positive.

Biosafety Precautions / 生物安全注意事项

All operations must be performed in a Class II biosafety cabinet.

需在II级生物安全柜中操作。

Waste must be strictly disinfected (e.g., 0.2% benzalkonium bromide).

废弃物需严格消毒 (如0.2%新洁尔灭)。

Strict adherence to biosafety protocols.

严格遵守生物安全规范。



Thank you!

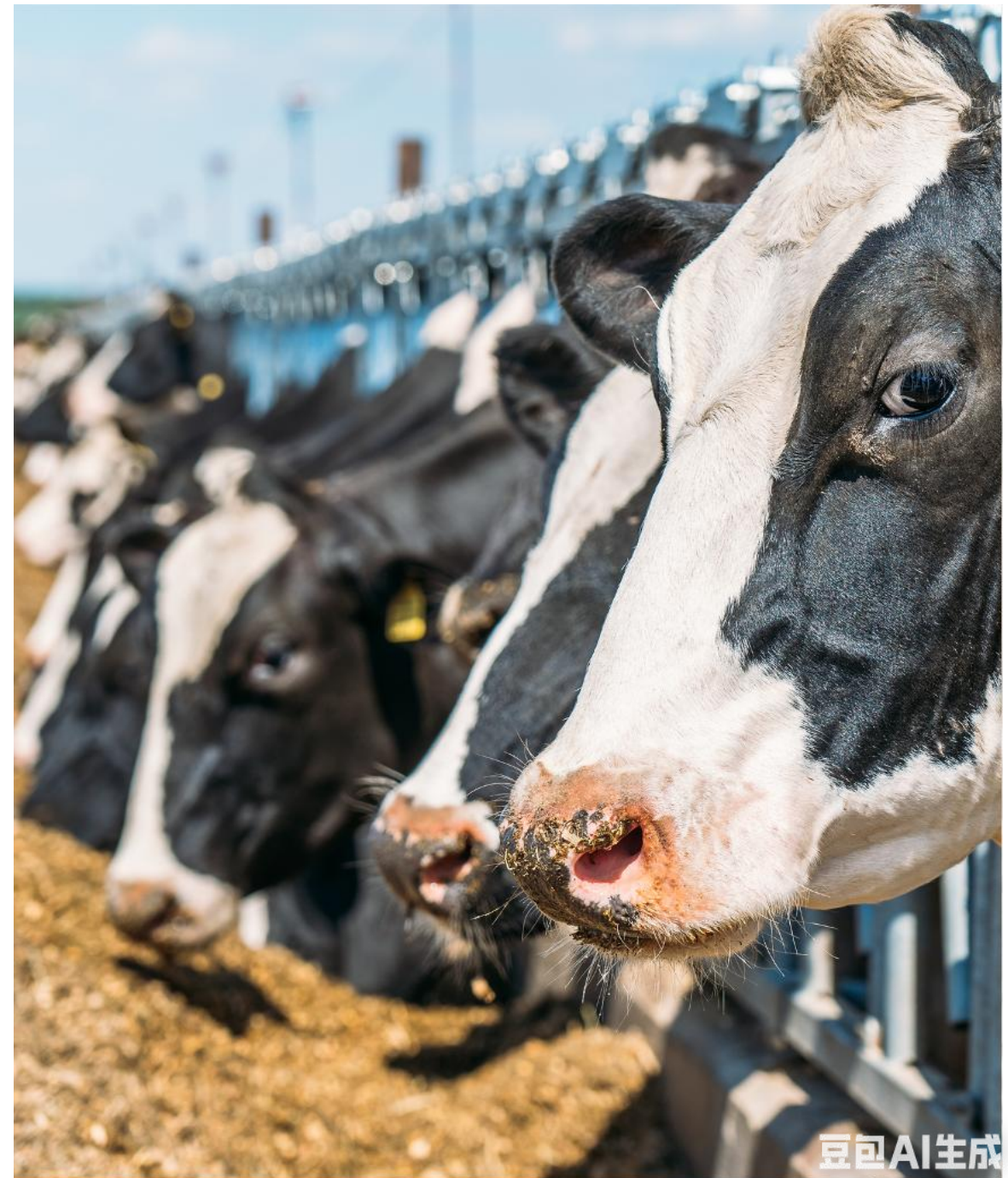
谢谢!

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