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: Molecular Diagnosis of Brucellosis
布鲁氏菌病的分子诊断

Country: China

Name of presenter: Dr. Junyi Hu

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Brucellosis: A Global Challenge

布鲁氏菌病：一个全球性的挑战

What is Brucellosis?

什么是布鲁氏菌病?

A zoonotic infectious disease caused by bacteria of the genus Brucella. Also known as "undulant fever" or "Malta fever".

由布鲁氏菌属细菌引起的人畜共患传染病，也被称为“波状热”或“马耳他热”。这类疾病可在动物与人类之间传播，是全球范围内重要的细菌性传染病之一。

Global Impact

全球影响

Endemic in many parts of the world, especially in Mediterranean countries, Middle East, Central Asia, and parts of Africa and Latin America. Estimated 500,000 new human cases annually.

该病在全球多地呈地方性流行，地中海国家、中东、中亚及非洲、拉美部分地区疫情尤为突出。据世界卫生组织估计，全球每年新增人类感染病例约50万例，对流行区公共卫生构成持续威胁。

Significance

重要性

A major cause of economic loss in the livestock industry. Severe chronic illness in humans if untreated, affecting multiple organs.

它是畜牧业经济损失的核心诱因之一，会导致牲畜流产、不孕等问题。对人类而言，若未及时规范治疗，将引发长期的严重慢性疾病，累及肝、脾、骨关节等多个器官系统，造成不可逆的健康损害。

Limitations of Conventional Diagnostic Methods

传统诊断方法的局限性

Bacterial Culture /细菌培养

- **Gold standard:**Considered the definitive method. / 被认为是确定性方法。
- **Time-consuming:**Can take 3-10 days for results. / 可能需要3-10天才能出结果。
- **Low Sensitivity:**Difficult to isolate the fastidious Brucella bacteria. / 难以分离挑剔的布鲁氏菌。
- **Hazardous:**Requires BSL-3 containment due to high infectivity. / 因高传染性，需BSL-3生物安全防护。

Serological Tests /血清学检测

- **Widely Used:**For screening (e.g., Rose Bengal, ELISA). / 用于筛查（如虎红试验，ELISA）。
- **Cross-reactivity:**May react with other bacterial infections. / 可能与其他细菌感染发生交叉反应。
- **Window Period:**Cannot detect early infection before antibody production. / 抗体产生前无法检测早期感染。
- **Persistent Antibodies:**Hard to distinguish active infection vs. past exposure/vaccination. / 难以区分活动性感染与既往感染或疫苗接种。

Overview of Molecular Diagnostics

分子诊断技术概述

Polymerase Chain Reaction (PCR) - The Cornerstone

聚合酶链反应 (PCR) - 分子诊断的基石

Application in Brucellosis
在布鲁氏菌病中的应用

Directly detects Brucella DNA from clinical samples (blood, tissue, etc.) at the molecular level, bypassing the need for culture.

直接从血液、组织等临床样本中检测布鲁氏菌DNA，在分子水平直接识别病原体，无需进行传统的细菌培养步骤，大幅缩短了检测周期。

Advantages / 核心优势

High Sensitivity & Specificity: Detects tiny amounts of DNA accurately.

高灵敏度和特异性：能够精准捕捉样本中微量的布鲁氏菌遗传物质，有效避免了传统检测方法的假阴性与交叉反应带来的误判。

Rapid Results: Available within hours.

快速出结果：相较于细菌培养需要数周的漫长等待，PCR技术数小时内即可出具检测报告，为临床及时制定治疗方案争取了宝贵时间。

Detects Non-Viable Bacteria: Identifies dead pathogens missed by culture.

可检测无活力细菌：即使是抗生素治疗后失去活性的布鲁氏菌，其DNA仍可被检测到，弥补了培养法无法检出死菌的缺陷，辅助判断感染残留。

Overview of Molecular Diagnostics

分子诊断技术概述

Conventional PCR
普通PCR

BCSP31-PCR • Nested PCR • AMOS PCR • Bruce-Ladder PCR

Real-time Quantitative PCR (qPCR)
实时荧光定量PCR (qPCR)

Loop-Mediated Isothermal Amplification (LAMP)
环介导等温扩增 (LAMP)

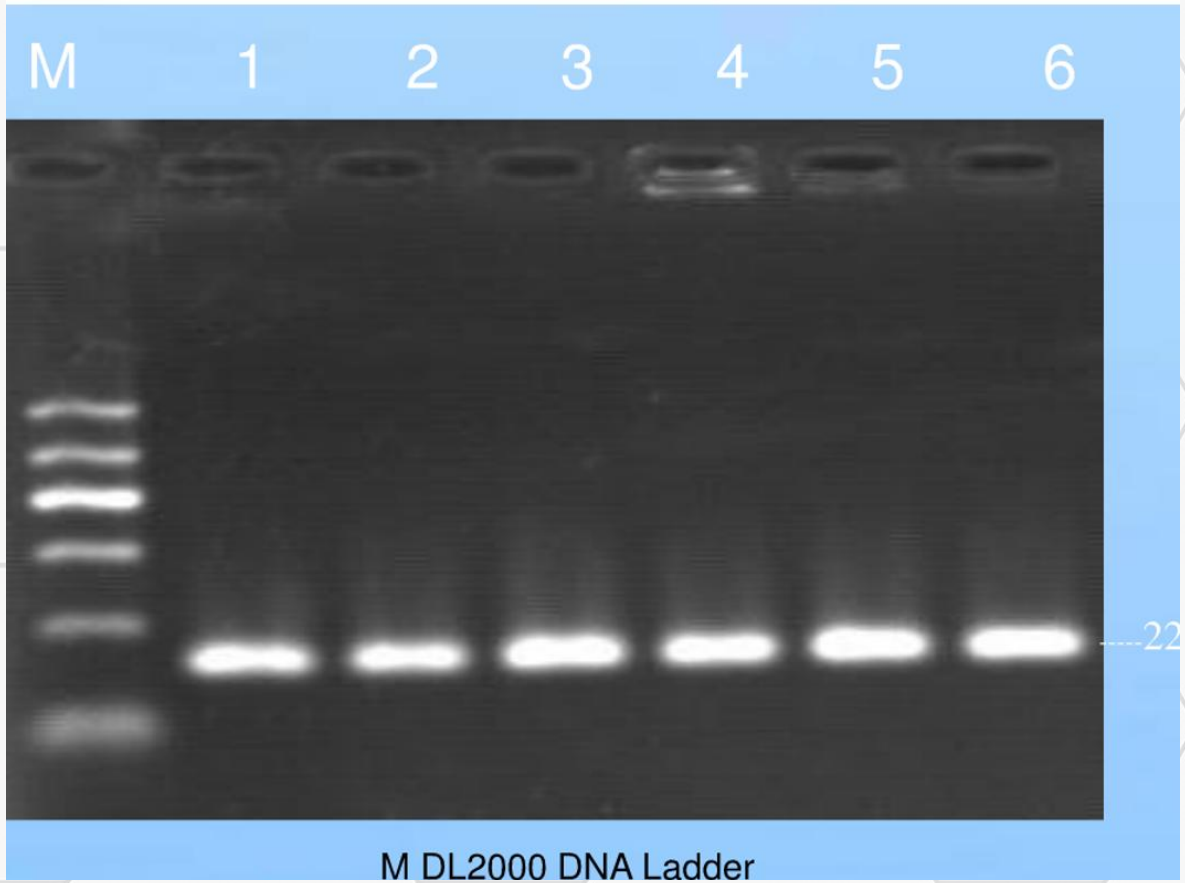
Droplet Digital PCR (ddPCR)
微滴式数字PCR (ddPCR)

Conventional PCR

BCSP31-PCR

- The**bcsp31**gene-based PCR assay for genus-level detection of*Brucella*spp.
- Forward Primer (B4):**5'-TGG CTC GGT TGC CAA TAT CAA-3'**
- Reverse Primer (B5):**5'-CGC GCT TGC CTT TCA GGT CTG-3'**
- Expected Amplicon Size:**223 bp**

Amplification results of BCSP31-PCR



Line 1~6: PCR products of

B. melitensis,

B. abortus, B. suis, B. ovis,

B. neotomae and B. canis.

Nested PCR: The Ultimate in Sensitivity

巢式PCR: 极致灵敏的检测方法

Principle /原理

Two rounds of PCR with outer & nested primers amplify DNA fragments sequentially, drastically boosting sensitivity & specificity.
（使用外侧与巢式引物进行两轮连续扩增，极大提升灵敏度与特异性）

Clinical Value /临床价值

Ideal for diagnosing stage1 infections; identifying pathogens in samples with very low bacterial loads where standard PCR may fail to detect the target DNA effectively.（非常适合诊断一期感染；在标准PCR可能无法有效检测目标DNA的极低细菌载量样本中鉴定病原体）

Nested PCR: The Ultimate in Sensitivity

巢式PCR: 极致灵敏的检测方法

Advantages /核心优势

- **Ultra-High Sensitivity:**Detects trace amounts of DNA (单拷贝/低载量，精准检测痕量DNA)
- **High Specificity:**Minimizes non-specific amplification (有效降低非特异性扩增的干扰)
- **Rapid Turnaround:**Results available in ~5 hours (仅需约5小时即可快速出具检测结果)

Limitations /局限性

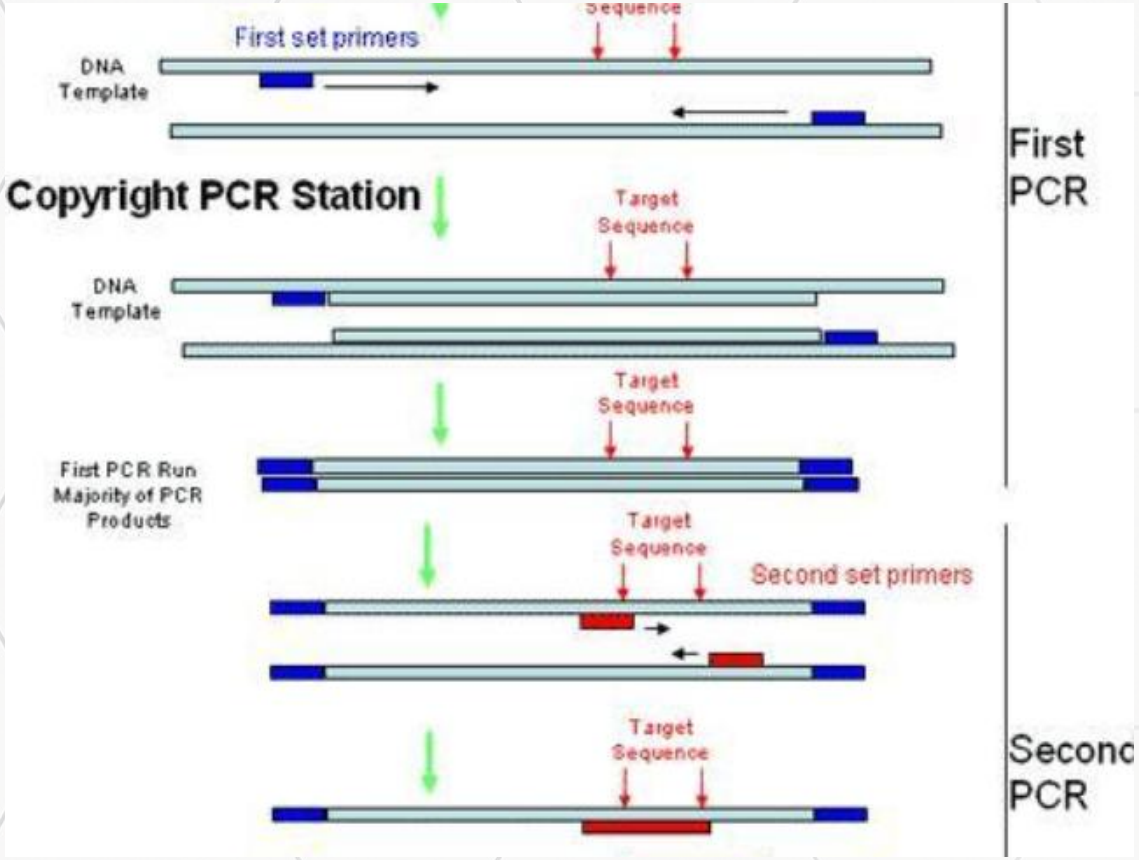
- **Contamination Risk:**Tube-opening increases carry-over risk (开盖操作流程易引发样本间的交叉污染)
- **Lack of Standardization:**No universal guidelines limit clinical use (缺乏通用标准化方案，一定程度限制临床推广应用)
- **Complexity:**More complex & labor-intensive than standard PCR (操作步骤比常规PCR更复杂，且耗时耗力)

Nested PCR: The Ultimate in Sensitivity

巢式PCR：极致灵敏的检测方法

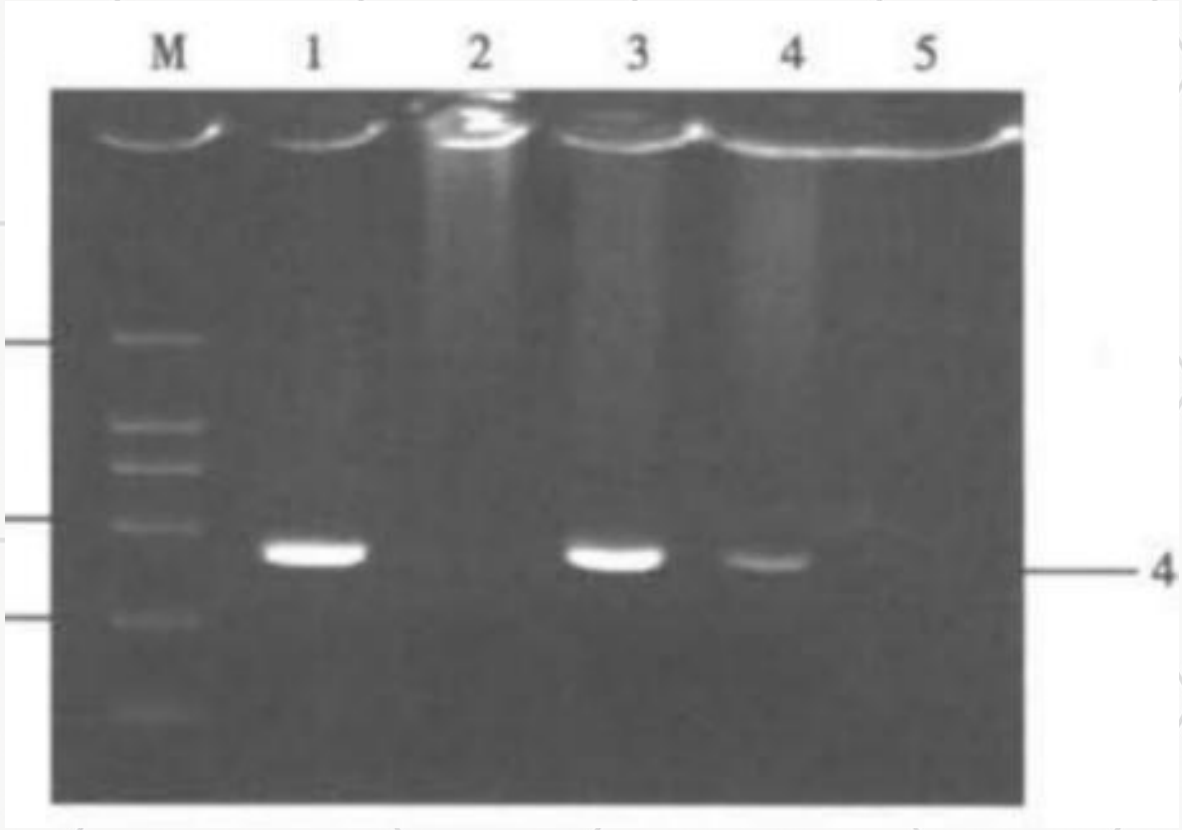
PCR for diagnosis of brucellosis in dairy cattle (NY/T 1467-2007)

The omp25 gene based nested PCR for detection of Brucella spp. at genus level.



Nested PCR: The Ultimate in Sensitivity

巢式PCR：极致灵敏的检测方法



Expected PCR product size: 419 bp

Lane 1: Positive control (Positive)
Lane 2: Negative control (Negative)
Lane 3&4: Clinical samples (Positive)
Lane 5: Clinical sample (Negative)

AMOS-PCR: A Classic Tool for Species Identification

AMOS-PCR：经典的菌种分型工具

Principle / 原理

AMOS-PCR is a multiplex PCR assay for the specific insertion sequence IS711 designed. The sequence exists in different Brucella genomes with varying copy numbers and locations, resulting in characteristic banding patterns after amplification.

（AMOS-PCR是一种多重PCR方法，针对特异性插入序列IS711设计。该序列在不同布鲁氏菌基因组中的拷贝数和位置不同，扩增后可产生特征性的条带大小）

Clinical Significance/临床意义

AMOS-PCR was the first species-specific multiplex PCR assay for the differentiation of Brucella, which was described by Bricker & Halling (1994).

AMOS-PCR: A Classic Tool for Species Identification

AMOS-PCR：经典的菌种分型工具

☑Advantages/核心优势

- Efficient Typing: Identifies major Brucella species (cow, sheep, pig, and ovine epididymal species).
- Highly practical: Widely used in clinical diagnosis and epidemiological investigations.

⚠ Limitations/局限性

- It is impossible to distinguish different biological forms of the same bacterial strain.
- It is unable to quantify the bacterial load in the sample.

AMOS-PCR: A Classic Tool for Species Identification

AMOS-PCR：经典的菌种分型工具

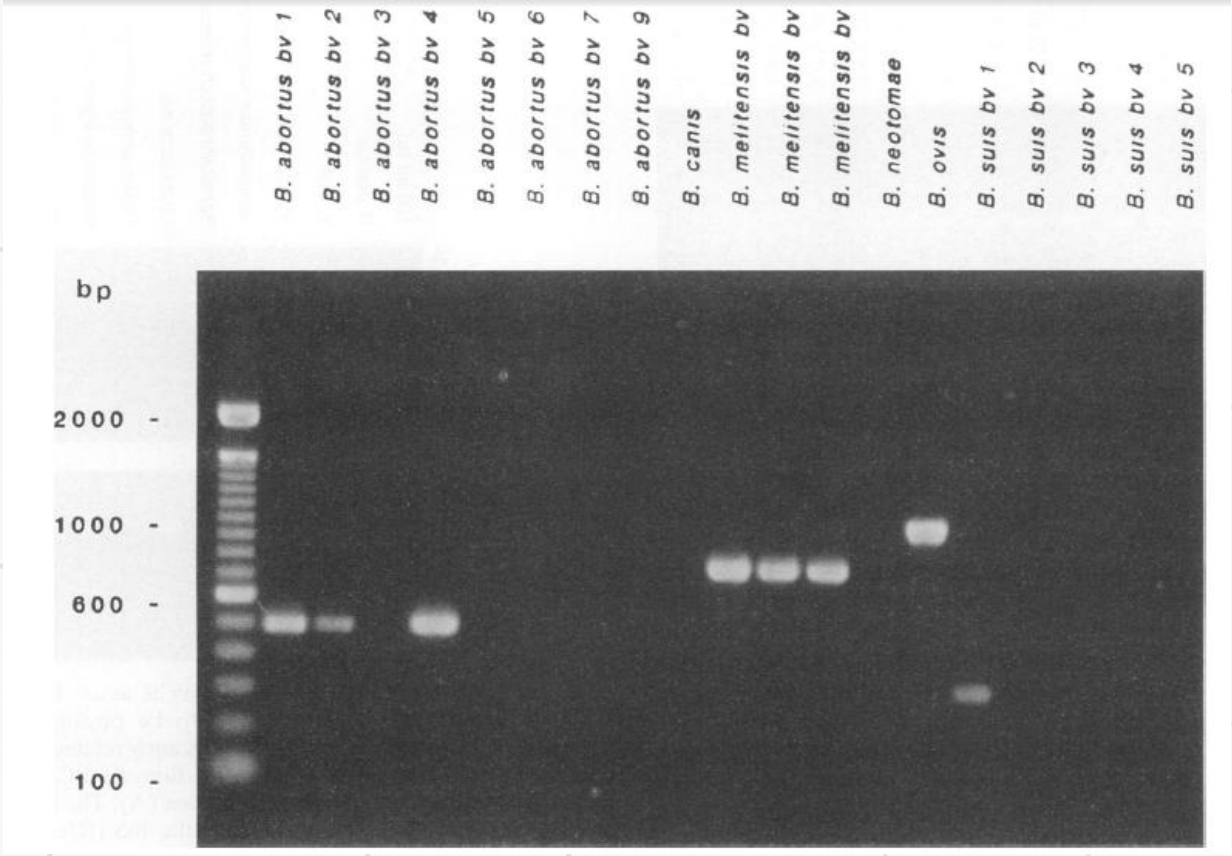
Expected PCR product size:

B. abortus(bv 1, 2 & 4):**498 bp**

B. melitensis(bv 1, 2, 3):**731 bp**

B. ovis:**976 bp**

B. suis(bv 1):**285 bp**



Bruce-Ladder PCR

Advantages of Bruce-Ladder PCR

- A multiplex PCR assay proposed for rapid and simple one-step identification of Brucella, simplifying the diagnostic workflow.
- Can identify and differentiate most Brucella species and key vaccine strains (S19, RB51, Rev.1) in a single experimental step, reducing time and labor.
- Possesses the capability to detect DNA from less common species including B. neotomae, B. pinnipedialis and B. ceti, expanding its applicability.

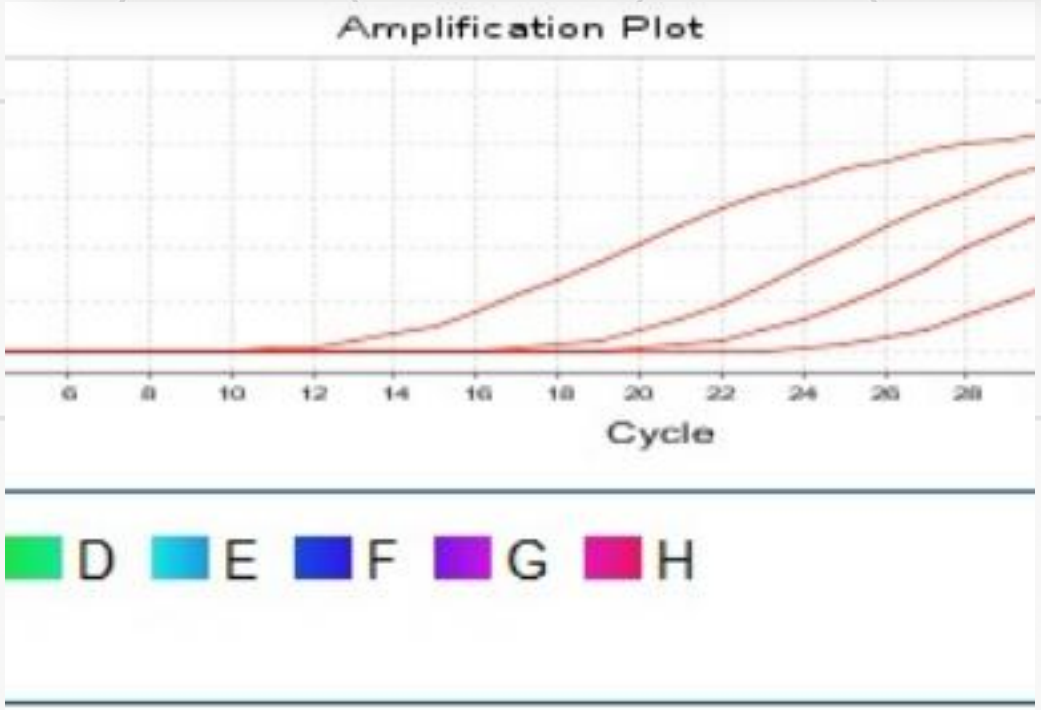
Expected PCR Products

- Bruce-Ladder PCR generates a characteristic banding pattern for different Brucella species and biovar, which acts as a unique "visual fingerprint" for each strain, enabling intuitive and reliable identification.
- This technique enables the differentiation of all major Brucella species in a single, standardized reaction, eliminating the need for multiple sequential tests and thus accelerating the diagnostic process.

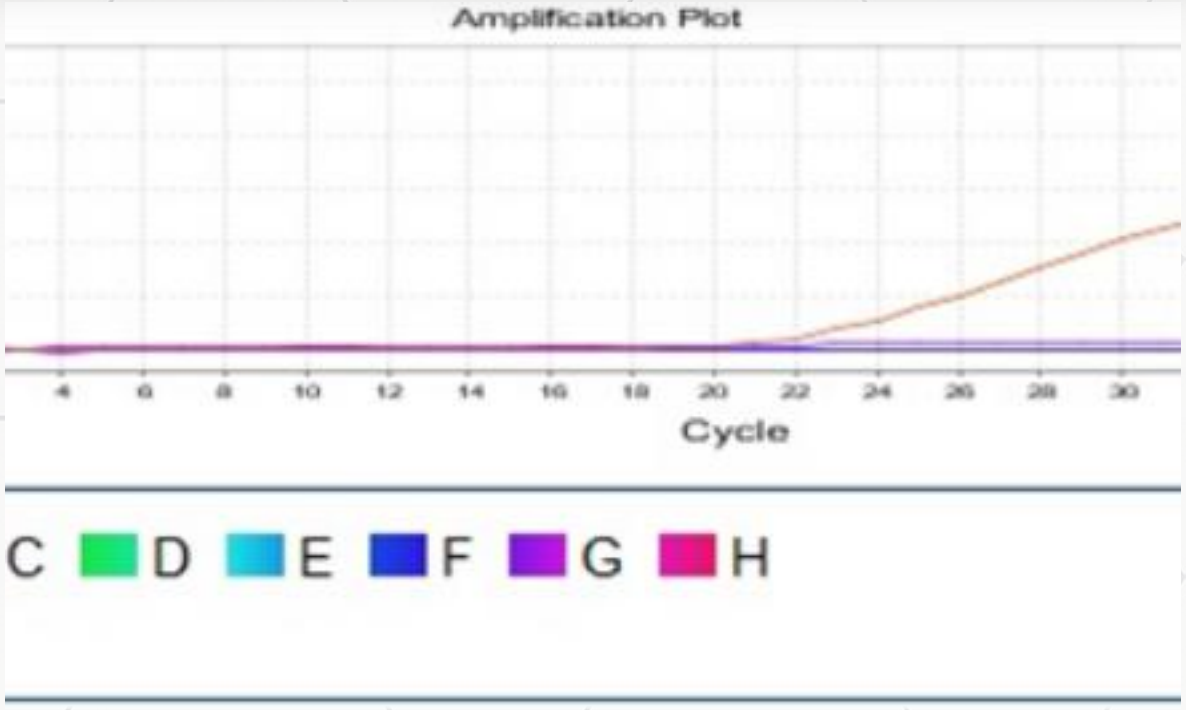
Real-time Quantitative PCR (qPCR) - Quantification and Real-time Monitoring

实时荧光定量PCR (qPCR) - 定量与实时监测

Specificity analysis



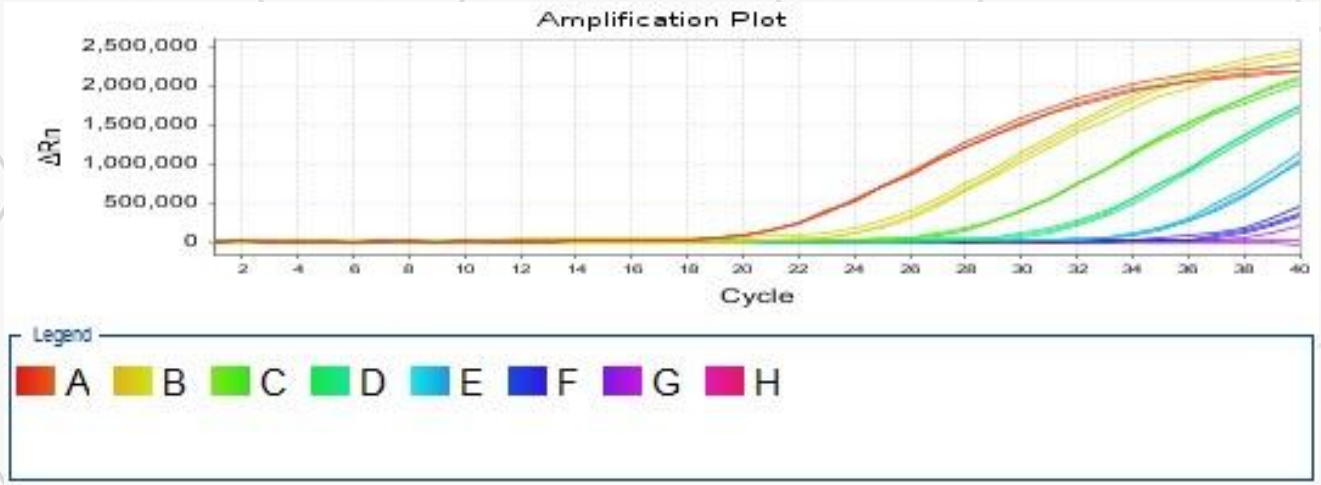
Amplification curve of*B. suis*,*B. melitensis*,*B. abortus*, and*B. canis*(from left to right)

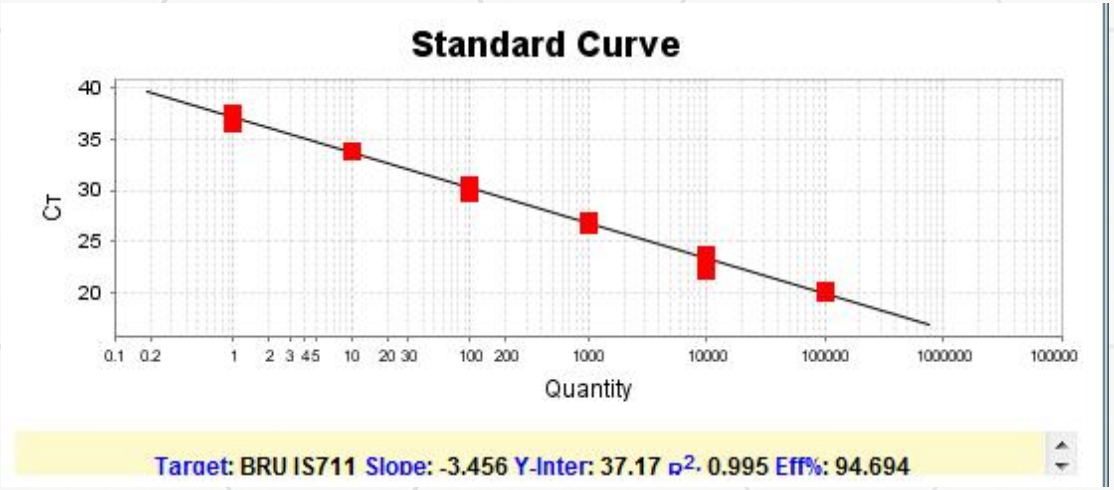


No amplification curve of common bacteria(*Escherichia coli, Salmonella, Staphylococcus aureus, bovine tuberculosis, Yersinia enteron, etc*)

Real-time Quantitative PCR (qPCR) - Quantification and Real-time Monitoring

实时荧光定量PCR (qPCR) - 定量与实时监测





This method was used to detect 6 bovine brucella nucleic acids in 10 fold serial dilutions (10^1-10^6), and the results showed that this method had good repeatability.

R^2 value=0.995，

Amplification efficiency: 94.69%

Loop-Mediated Isothermal Amplification (LAMP) - Rapid Detection at Constant Temperature

环介导等温扩增 (LAMP) - 等温条件下的快速检测

Core Mechanism
核心作用机制

01 / Principle / 原理

- Invented by Notomi in 2000.
- Amplifies DNA under isothermal conditions (60-65°C), no thermal cycler.
- Uses 4 specially designed primers recognizing 6 distinct sequences.

由Notomi于2000年发明。在等温条件下(60-65°C)扩增DNA，无需热循环仪。使用4个特殊设计的引物，识别6个不同序列。

Key Benefits
技术核心优势

02 / Advantages / 优点

High Specificity & Sensitivity: Due to multiple primer binding sites.
Rapid & Efficient: Results available within 1 hour.
Simple & Cost-Effective: No expensive equipment; visual detection possible.
Suitable for POC Testing: Ideal for resource-limited settings.

高特异性与敏感性：依托多个引物结合位点实现精准识别；快速高效：1小时内即可获得检测结果；简单且成本低：无需昂贵仪器，可通过目视完成结果判定；适用于POC检测：是资源匮乏环境下即时检测的理想技术方案。

Limitations of LAMP Technology

LAMP技术的局限性

Amplicon Length Limitation

The optimal amplicon size is between 30-200 bp, which can limit target selection.

扩增片段长度限制

最佳扩增片段大小在30-200 bp之间，这可能限制目标序列的选择。

Detection of Non-specific Products

Difficult to distinguish between specific and non-specific amplicons using simple visual detection methods.

非特异性产物检测

使用简单的目视检测方法，可能难以区分特异性和非特异性扩增产物。

Risk of Contamination

The high efficiency of LAMP makes it highly susceptible to aerosol contamination, leading to false positives.

污染风险

LAMP的高效率使其极易受气溶胶污染，导致假阳性结果。

Primer Design Complexity

Designing the six-primer set can be more complex and time-consuming compared to PCR.

引物设计复杂性

与PCR相比，设计六引物组可能更复杂、更耗时。

Droplet Digital PCR (ddPCR) - A New Dimension in Absolute Quantification

微滴式数字PCR (ddPCR) - 绝对定量的新维度

01 / Principle |原理

Core steps: Partition the PCR reaction mixture into thousands of independent droplets (sample partitioning), with each droplet serving as an individual reaction chamber for single-molecule amplification.
Quantification method: By counting the number of positive and negative droplets, the initial concentration is calculated based on the Poisson distribution principle, achieving absolute quantification without the need for a standard curve.

- **核心步骤:**将PCR反应体系分割成成千上万个独立的液滴（样本分区），每个液滴作为独立反应腔进行单分子扩增。
- **定量方式:**根据计数阳性液滴和阴性液滴的数量，依据泊松分布原理计算初始浓度，实现无需标准曲线的绝对定量。

02 / Key Advantages |核心优势

- **Ultra-high Sensitivity:**Capable of detecting target molecules down to a single copy per sample.
- **High Tolerance:**Strong resistance to PCR inhibitors in complex sample matrices, providing robust anti-interference performance.
- **High Performance:**Achieves accurate absolute quantification without the need for standards, with excellent repeatability.
- **超高灵敏度：**可对样本中低至单个拷贝的目标分子进行精准检测，突破传统检测下限。
- **高耐受性：**对复杂样本基质中的PCR抑制物具备极强抵抗力，抗干扰能力突出，适配多样本类型。
- **卓越性能：**无需标准品即可获得精确的绝对定量结果，且实验重复性极佳，数据稳定性强。

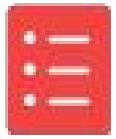
Limitations of ddPCR Technology

ddPCR技术的局限性

Longer Detection Time

检测时间较长

The process of droplet generation and reading adds to the overall time compared to qPCR.
与qPCR相比，液滴生成和读取过程增加了整体检测时间。



Lower Throughput

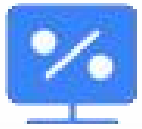
通量较低

Less suitable for high-volume screening of large numbers of samples.
不太适合对大量样本进行高通量的快速筛查工作。

Higher Cost

成本较高

The specialized equipment and consumables for ddPCR can be more expensive.
ddPCR所需的专用设备和耗材相对成本较高。



Data Analysis Complexity

数据分析复杂性

Requires specialized, complex software for droplet analysis and precise quantification.
需要专业、复杂的软件对液滴信号进行分析和精确定量。

Laboratory Biosafety

High Risk Pathogen

Brucella is recognized as one of the most frequent pathogens causing laboratory-acquired infections (LAIs), posing a significant occupational hazard.

Multiple Transmission Routes

It can spread easily through direct skin/mucous membrane contact, accidental ingestion, or inhalation of aerosols generated during sample processing.

High Bacterial Loads

Clinical specimens (e.g., blood, tissues) for nucleic acid extraction often harbor a large concentration of live Brucella bacteria.

Strict adherence to biosafety protocols is non-negotiable to protect personnel and the environment.

Biosafety requirements for brucella related operations

Large number of live bacteria operation:**BSL-3**

Animal infection test:**ABSL-3**

Sample testing:**BSL-2**

Experiments with non-infectious materials:**BSL-1**

Thank you!

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Connect with us for more information and updates on animal health initiatives across the Asia-Pacific region.