

ELISA for Brucellosis Diagnosis

Principles and Practical Applications

CHINA

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Agenda

01

Introduction to ELISA

- Definition, Core Principles
- Overview of Major ELISA Formats

02

Practical Application

- Step-by-Step Protocol:
Indirect ELISA
- Step-by-Step Protocol:
Competitive ELISA

03

Critical Considerations

- Critical factors

1.1 What is ELISA?

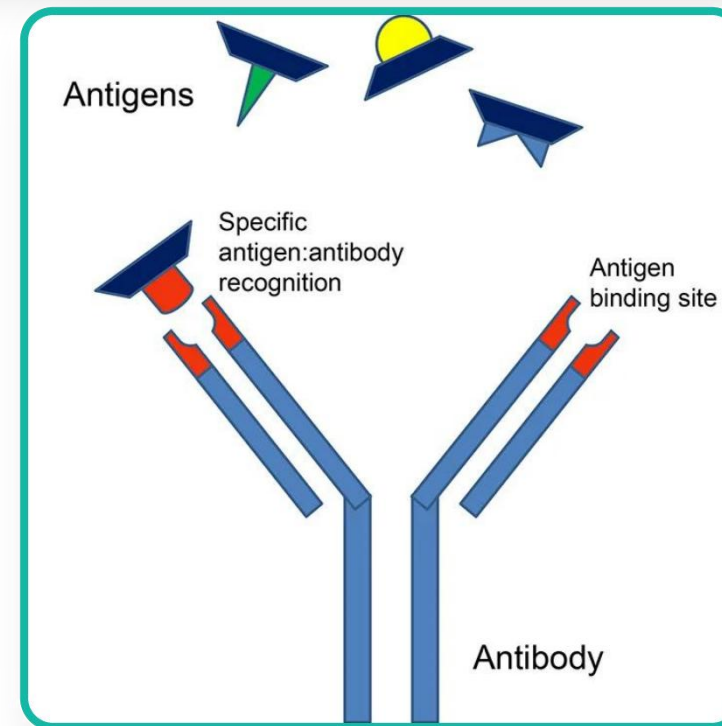
Enzyme-Linked Immunosorbent Assay

ELISA is a powerful immunoassay technique that combines the *specificity* of antigen-antibody reactions with the *sensitivity* of enzyme catalysis. It is used to detect and quantify substances such as antibodies, antigens, and proteins in a sample.

Core Principles

The magic of ELISA lies in two principles working together.

- **Highly Specific Binding:** Antibodies bind specifically to their target antigens, ensuring accuracy.
- **Enzymatic Amplification:** We attach an enzyme to one of these molecules. This enzyme acts like an amplifier; when a substrate is added, it creates a visible signal, the strength of which tells us how much target was present in the original sample.



1.2 Major ELISA Formats

01 / Indirect ELISA

Use: Detects antibodies in a sample. *It uses enzyme-labelled anti-species antibody to detect sample antibodies.*

Method:

Antigen is coated onto the plate. Antibodies present in the sample bind to the coated antigen. Finally, an enzyme-labeled secondary antibody is added to bind to the sample antibodies, enabling detection.

02 / Competitive ELISA

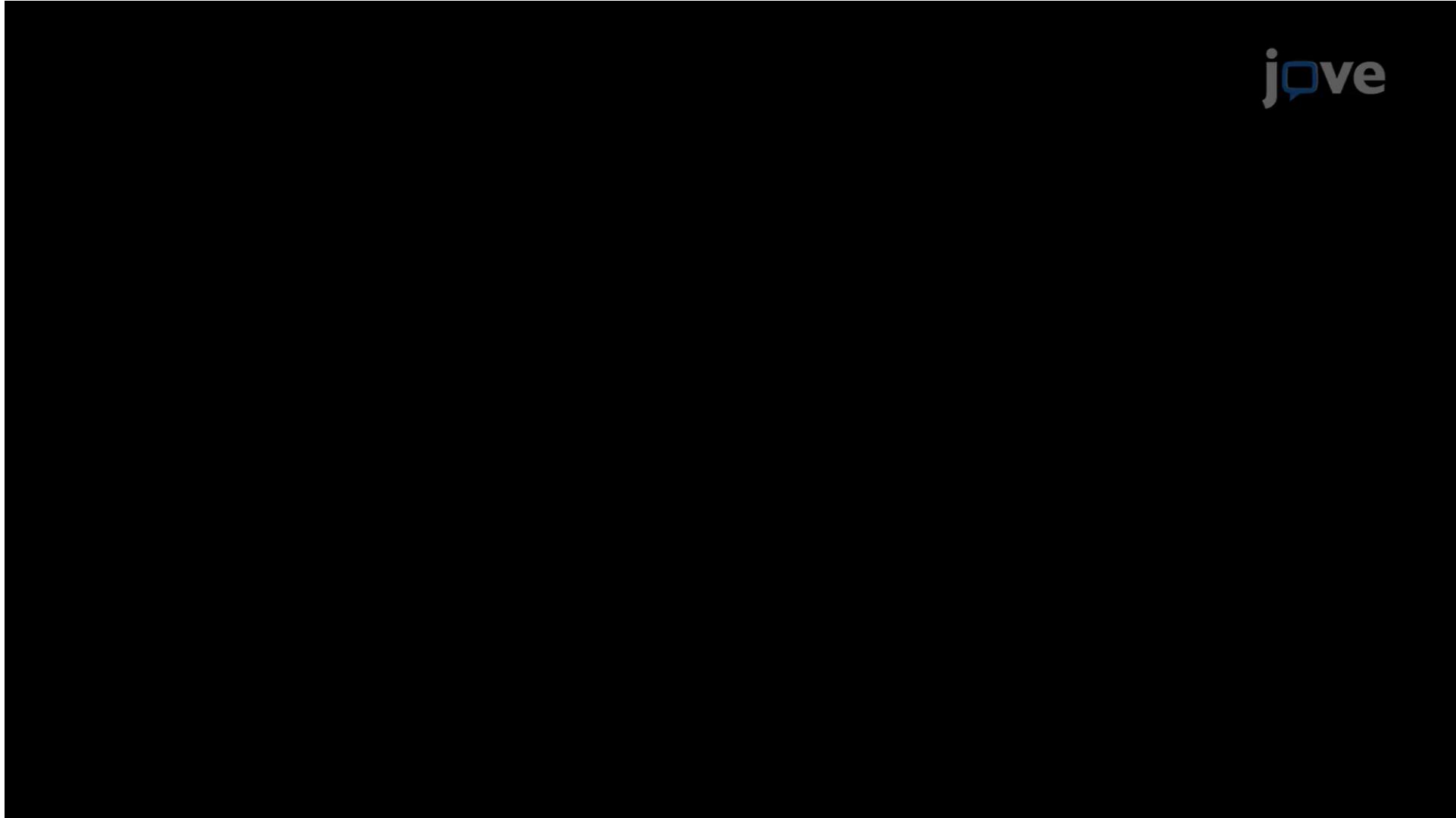
Use: Detects antigens or antibodies. *It uses a labelled monoclonal antibody competing with sample antibodies for antigen binding, offering greater species-independence.*

Method:

Sample analyte competes with the specific antibody for binding sites on a coated antigen. *A higher concentration of analyte in the sample leads to less labeled analyte binding, resulting in a weaker signal (inverse relationship).*

Let's summarize the key differences between the two formats. Indirect ELISA uses layered binding and has a direct signal correlation, making it highly sensitive and ideal for screening. Competitive ELISA uses a competition principle with an inverse signal correlation, making it better for quantification and often more specific. Your choice depends on whether you need high sensitivity for screening or accurate quantification for confirmation.

Part 2: Practical Application



Part 3: Critical Considerations

Obtaining accurate and reproducible results in ELISA requires strict adherence to protocols and careful attention to several critical factors. This section highlights the key steps and general precautions for success.



Washing Steps

Prevent high background noise and non-specific binding by ensuring thorough, consistent wash cycles.



Incubation Conditions

Strictly follow time and temperature guidelines to guarantee optimal antibody-antigen binding kinetics.



Accurate Pipetting

Minimize well-to-well variation and ensure precision with calibrated pipettes for consistent volume delivery.



Reagent Handling

Avoid contamination and maintain stability by properly storing and mixing all assay reagents.



Sample Quality

Use fresh, properly processed samples to prevent degradation or interference that skews readings.



Safety Precautions

Wear personal protective equipment (PPE) and follow biohazard protocols for handling biological materials.

Thank You

Q & A

Feel free to ask any questions

