

Presentation Title:
**Laboratory Diagnostic Techniques for
Brucellosis: Serum Agglutination Test
(Macro-method)**

Country- P.R. China

Name of presenter-Dr. Zhang Xiaoqian

Designation of the presenter-Government Veterinary Surgeon

2nd WOAHA Regional Training Workshop on Brucellosis Diagnosis

Beijing, P.R. China, 26 - 29 May 2026



What is the serum agglutination test?

- The serum agglutination test is a laboratory test which involves the serial dilution and testing of a serum sample. The test is performed by the preparation of different dilutions of a serum sample at increasing concentrations for serum titer determination.

CONTENTS

01

**Principle of Serum
Agglutination Test**

02

**Experimental
Operation Procedure**

03

**Interpretation and
Analysis of Results**

04

**Advantages of
the Method**

05

**Limitations of the
Method and
Directions for
Improvement**

06

**Clinical Applications
and Prospects**



PART 01

Principle of Serum Agglutination Test



Mechanism of antigen-antibody reaction



01

The binding process of antigen and antibody

In the serum agglutination test, the specific binding of antigen and antibody is the basis. When a reagent containing Brucella antigen is mixed with the serum of an infected animal, if there are antibodies against the antigen in the serum, the two will specifically bind to form a visible agglutination phenomenon. This process relies on the molecular structure complementarity of antigens and antibodies, ensuring the high specificity of the test.

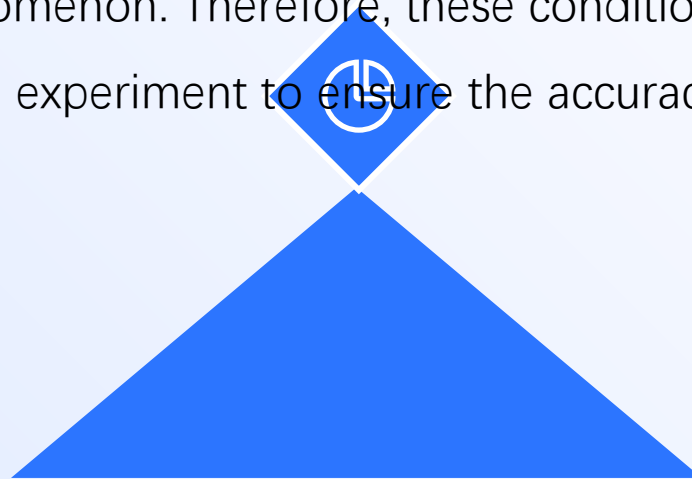


Mechanism of antigen-antibody reaction

02

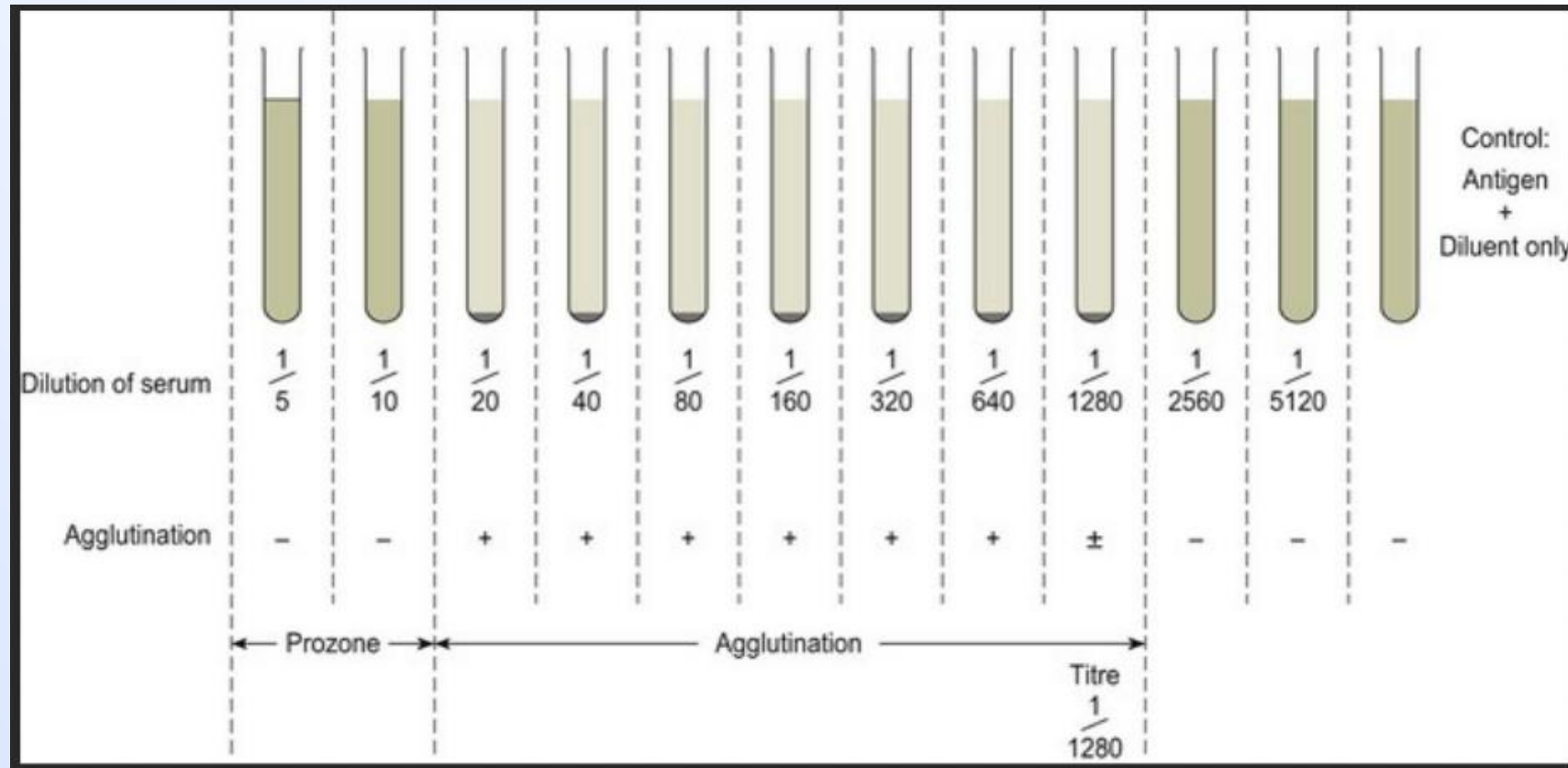
Conditions for the formation of agglutination phenomenon

The formation of the agglutination phenomenon requires appropriate conditions, including a suitable antigen-antibody ratio, pH value, and ionic strength. When the ratio of antigen to antibody is appropriate, the formed complexes can aggregate into visible agglutination clumps. In addition, temperature and reaction time also affect the visibility of the agglutination phenomenon. Therefore, these conditions must be strictly controlled during the experiment to ensure the accuracy of the results.



The prozone phenomenon of SAT

False negative at low dilutions due to antibody excess;
agglutination appears at higher dilutions





PART 02

Experimental Operation Procedure



Preparation of Reagents and Samples

01



Selection and Preparation of Reagents

Before conducting a serum agglutination test, it is necessary to select **an appropriate Brucella antigen reagent** and prepare it correctly in accordance with the instructions. The quality of the antigen reagent directly affects the accuracy of the test results, so verified high-quality reagents should be chosen. In addition, **auxiliary reagents** such as distilled water and serum sample diluent need to be prepared to ensure the smooth progress of the experiment.

Preparation of Reagents and Samples

02



Collection and Processing of Samples

The collected serum needs to be stored at an appropriate temperature and properly diluted before the experiment. The dilution ratio should be adjusted according to the experimental requirements and the antibody level of the samples to ensure a suitable ratio for the antigen-antibody reaction and enhance the reliability of the test results.

General Procedure of SAT

- The test is performed by the preparation of different dilutions of a serum sample at increasing concentrations for serum titer determination.

General Procedure of SAT

- Specifically defined amounts of antigen are then added to each of these tubes and incubated at 37°C for approximately 24 hours.
 - After incubation, the tubes are then qualitatively observed to detect the presence of clumps at the bottom of each test tube.
- This test is analysed using the 50% agglutination method to obtain the titer about its relative agglutination amount.

General Procedure of SAT

- Doubling dilution of the test serum, starting from 1:12.5, was done with 0.5% phenol saline in tubes.
- Antigen bottle was shaken well and brought to room temperature.

General Procedure of SAT

- Equal amount of (0.5 mL) Brucella antigen was added to each tube. The contents of each tube were mixed by rolling in between the palms. The dilutions ranged from 1:25 in the first tube, to 1:400 in the fifth tube. The tubes were incubated at 37°C for 24 hours.
- Parallel to set of test sera , a set of 5 tubes was used with antigen in 0.5% phenol saline for comparing the result of the test sample. Standard antigen nephelometric tubes were also incubated at 37°C for 24 hours.

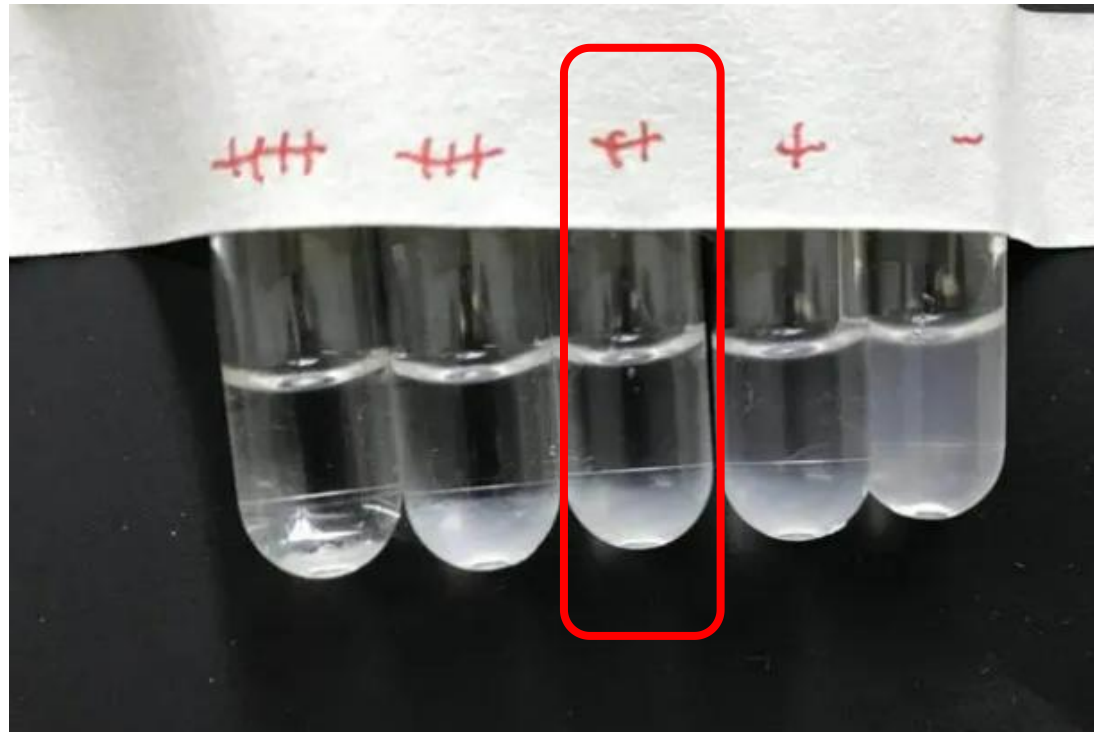
Dilution of standard antigen nephelometric tube

Tube	0.5% phenol saline (μL)	Antigen (μL)	Degree of agglutination
Tube 1	0	1000	None
Tube 2	250	750	25%
Tube 3	500	500	50%
Tube 4	750	250	75%
Tube 5	1000	0	100%

Antigen Standardisation

1000 IU standard positive serum is used for calibration

The calibrated SAT antigen reacts with 1:1000 diluted standard positive serum, and "++" agglutination occurs (≥ 30 IU/mL in some WOAHA guidelines is indicated positive)





PART 03

Interpretation and Analysis of Results



Observation and Judgment of Agglutination Phenomenon

Grading Standards for Agglutination Phenomenon

In serum agglutination tests, the grading of agglutination phenomena is an important basis for judging results. Generally, agglutination phenomena can be divided into four grades: negative, weakly positive, positive, and strongly positive. Negative indicates no agglutination or only a trace amount of agglutination, while strongly positive indicates the formation of obvious agglutination clumps. Accurate grading judgment helps evaluate the degree of infection and the progression of the animal condition.

Observation and Judgment of Agglutination Phenomenon

Interpretation of the Clinical Significance of Results

The interpretation of test results should be combined with the animal clinical symptoms and epidemiological history. A positive result suggests that the animal may be infected with Brucella, and further etiological testing is required to confirm the diagnosis. A negative result may indicate that the animal is not infected or is in the early stage of infection, with antibody levels not yet elevated. Therefore, the interpretation of results needs to take various factors into account to ensure the accuracy of the diagnosis.

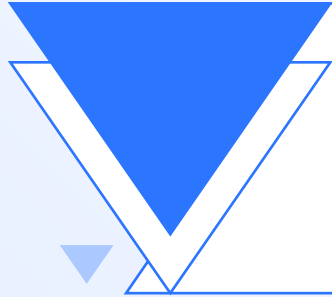


PART 04

Advantages of the Method



Analysis of the Advantages of the Method



High specificity and sensitivity

The serum agglutination test has **high specificity and sensitivity**, and can accurately detect **Brucella antibodies in animal serum**. This method is easy to **operate with intuitive results**, making it **suitable for large-scale screening and clinical diagnosis**. In addition, **the test cost is relatively low**, which makes it suitable for promotion and application in areas with limited resources, providing an effective means for the prevention and control of brucellosis.



PART 05

Limitations of the Method and Directions for Improvement

Analysis of Limitations

Possibility of cross-reactivity

Although the serum agglutination test has high specificity, cross-reactivity may occur in some cases, leading to false-positive results. This is mainly due to the similarity in antigens among different bacteria or the presence of other cross-reactive antibodies in the animal serum. Therefore, caution should be exercised when interpreting the results, and if necessary, it should be verified in combination with other detection methods to improve the accuracy of diagnosis.

Dependence on operating techniques

The results of the serum agglutination test are greatly affected by operating techniques, such as the ratio of antigen to antibody and reaction conditions. Improper operation may lead to result deviations and affect the accuracy of diagnosis. Therefore, laboratory personnel need to receive professional training and skillfully master experimental operation techniques to ensure the reliability of test results. In addition, the establishment of standardized operating procedures is also an important measure to improve the quality of the test.

Disadvantages

- **Disadvantages:**

Tests false-positive or false-negative, prozone phenomenon,
not used for the purpose of integrational trade.



PART 06

Clinical Applications and Prospects



Current status of clinical application

01

Application in clinical diagnosis

Currently, the serum agglutination test has been widely applied in the clinical diagnosis of brucellosis, especially in endemic areas and among high-risk populations. This method can quickly screen out suspected cases, providing a basis for subsequent etiological testing and treatment. In addition, it is also used to monitor animal treatment effects and disease changes, helping adjust treatment plans and improve therapeutic outcomes.

02

Application in epidemiological surveys

The serum agglutination test also plays an important role in epidemiological surveys of brucellosis. Through large-scale screening, the distribution and transmission trends of the disease can be understood, providing a scientific basis for formulating prevention and control measures. In addition, this method is used to evaluate the effectiveness of prevention and control measures, monitor the dynamic changes of the disease, and provide support for public health decision-making.

Thank you!

*China Institute of Veterinary Drug Control
National Reference Laboratory for Animal Brucellosis
No. 33, Qingfeng West Road, Daxing District
Beijing, 102600
[P.R. China](#)
zhangxiaoqian0329@126.com*

