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: Complement Fixation Test (CFT)

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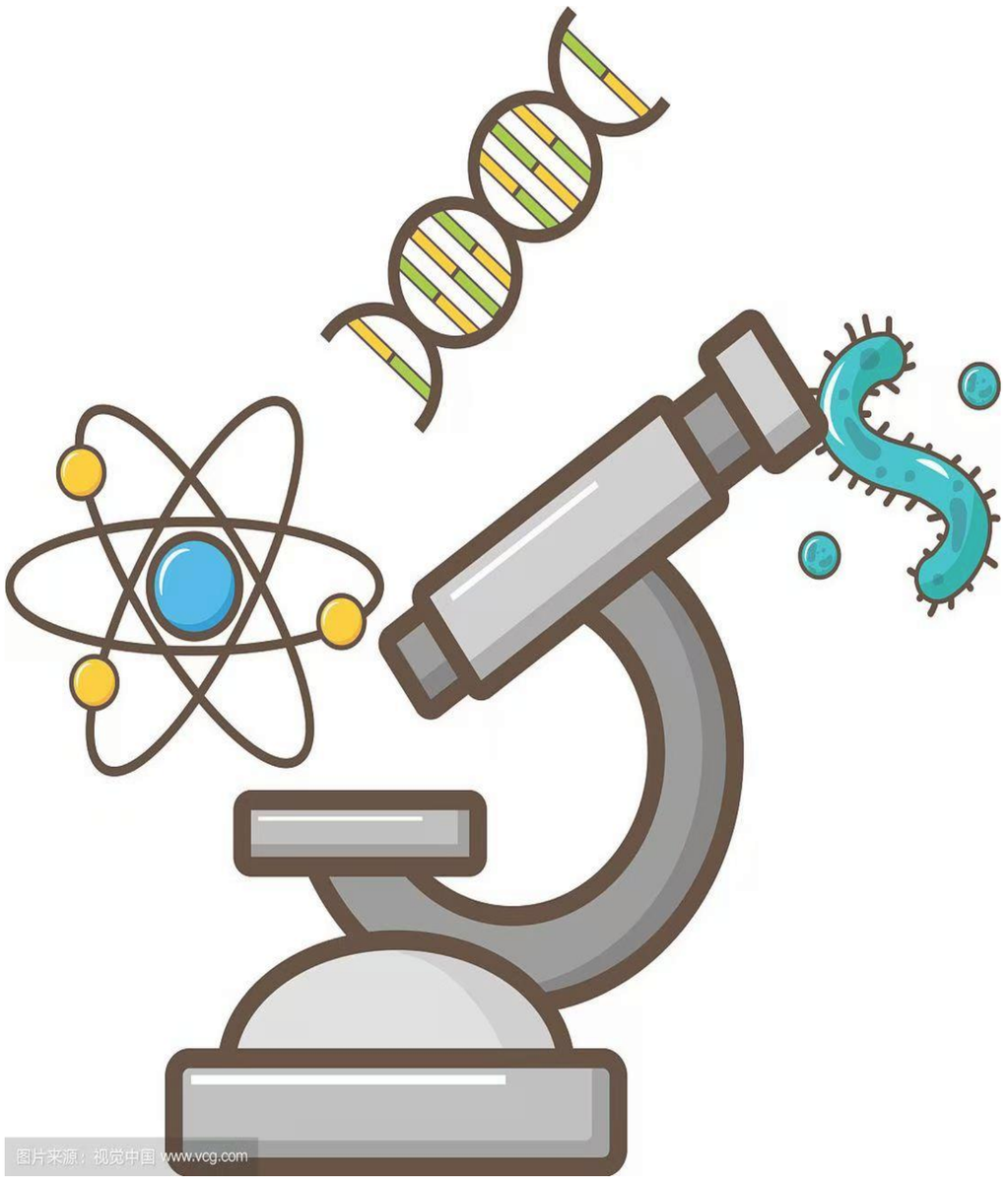

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Introduction

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Overview of CFT

Definition

Complement Fixation Test (CFT) is a serological test used to detect the presence of specific antibodies by observing the interaction between antigen-antibody complexes and complement proteins. It is widely used in diagnosing various infectious diseases and autoimmune conditions.

Historical Background

The CFT was first developed in the early 20th century. It revolutionized the diagnosis of diseases like brucelosis and tuberculosis by providing a reliable method to detect antibodies in infected animals' sera. Over the years, it has been refined and adapted for numerous clinical applications.

Importance in Diagnosis

CFT remains a crucial tool in clinical microbiology. It helps in confirming infections caused by pathogens that are difficult to culture, such as certain viruses and intracellular bacteria. Its ability to detect specific antibodies makes it invaluable for early diagnosis and treatment planning.

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Principle of CFT

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Antigen-Antibody Interaction

Formation of Complexes

When antigens and antibodies are mixed, they form antigen-antibody complexes. These complexes can activate the complement system, leading to a cascade of reactions that result in the consumption of complement proteins.

Complement Activation

The complement system is a series of proteins that enhance the immune response. In CFT, the presence of antigen-antibody complexes triggers the activation of complement, which is then consumed in the process.

Indicator System

An indicator system, typically sheep red blood cells (SRBC) coated with anti-SRBC antibodies, is used to detect the presence or absence of complement. If complement is consumed by the antigen-antibody complexes, the SRBC will not lyse, indicating a positive test.

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Interpretation of Results

Positive Test

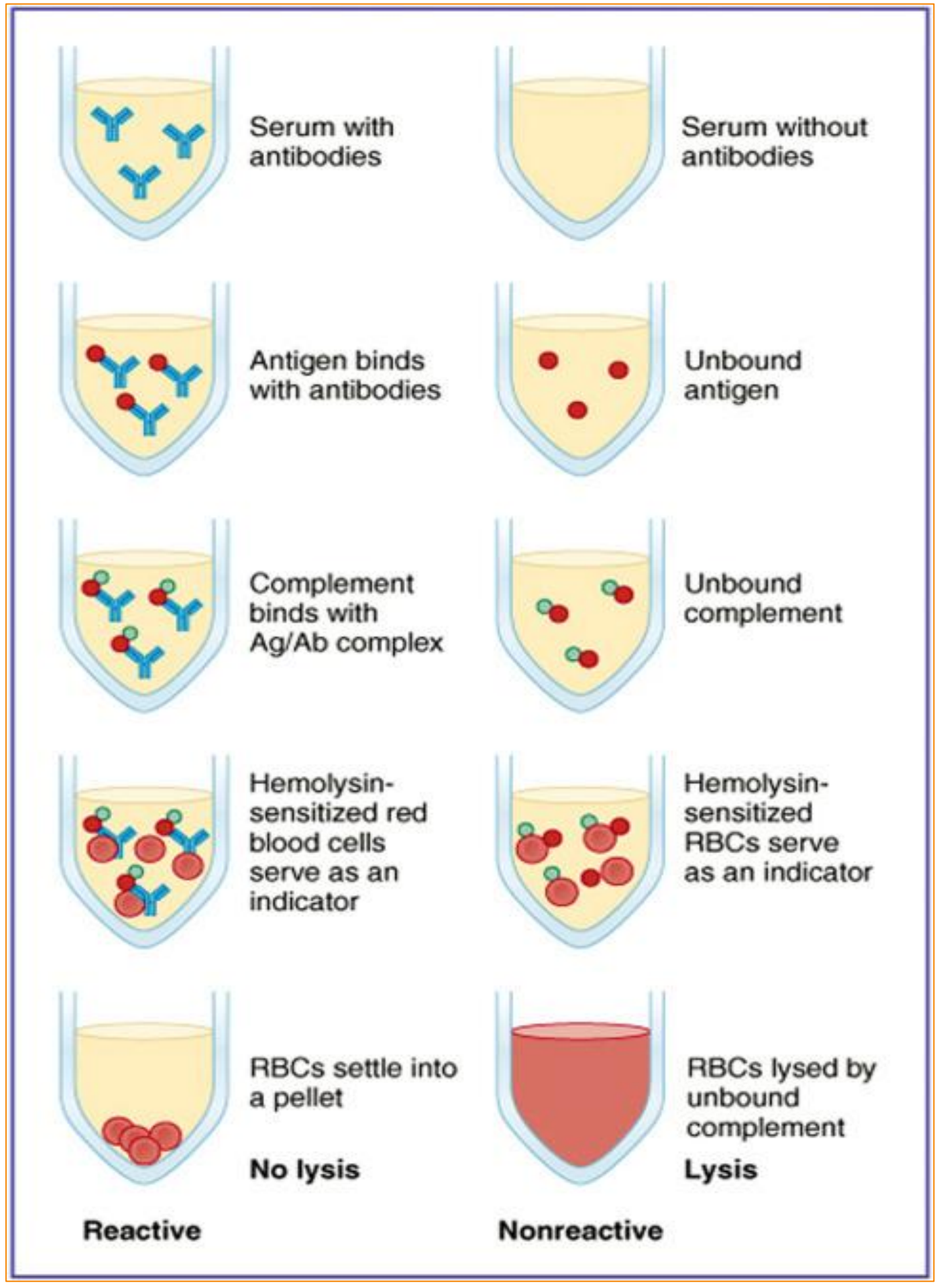
A positive CFT result is indicated by the absence of hemolysis of the indicator SRBC. This means that the complement has been fixed by the antigen-antibody complexes, confirming the presence of specific antibodies in the test serum.

Negative Test

A negative result is shown by the lysis of SRBC, indicating that complement is still available and no specific antibodies were present to form complexes and fix the complement.

Titration and Quantification

Titration of the test serum can be performed to determine the titer of specific antibodies. This provides a quantitative measure of the immune response and helps in assessing the severity of infection or disease progression.



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Components of CFT

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Antigen

Source and Preparation

01

The antigen used in CFT must be specific to the pathogen or condition being tested. It can be purified from natural sources or produced using recombinant DNA technology. Proper preparation ensures that the antigen is stable and retains its immunoreactivity.

Role in Test

02

The antigen is mixed with the infected animals' serum to allow the formation of antigen-antibody complexes. Its specificity is crucial for accurate test results.

Quality Control

03

Quality control measures are essential to ensure the consistency and reliability of the antigen. This includes verifying its purity, concentration, and ability to bind antibodies effectively.

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Antibody

Types of Antibodies

CFT primarily detects IgG and IgM antibodies. These antibodies are produced by the immune system in response to infection or immunization and can bind specifically to the antigen.

Detection in Test

The presence of antibodies in the infected animals serum is indicated by the fixation of complement. The test is designed to detect even low levels of specific antibodies, making it highly sensitive.

Clinical Significance

The type and titer of antibodies detected can provide valuable information about the stage of infection and the immune response. For example, IgM antibodies are typically produced early in an infection, while IgG antibodies indicate a more established or past infection.

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Complement

01

Source and Purification

Complement proteins are usually obtained from fresh guinea pig serum. The serum is processed to ensure the complement is active and free from contaminants that could interfere with the test.

02

Role in Test

The complement system is activated by antigen-antibody complexes. Its consumption is the key indicator of a positive test result. The complement must be titrated to an optimal concentration to ensure accurate results.

03

Stability and Storage

Complement is sensitive to temperature and must be stored at appropriate conditions to maintain its activity. Proper handling and storage are critical to ensure the reliability of the test.

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Indicator System

Choice of Indicator

Sheep red blood cells (SRBC) are commonly used as the indicator system. They are coated with anti-SRBC antibodies to make them sensitive to complement-mediated lysis.

Sensitivity and Specificity

The indicator system must be highly sensitive to detect even small amounts of complement. It should also be specific, with minimal background lysis in the absence of complement fixation.

Preparation and Validation

The SRBC must be prepared under sterile conditions and validated to ensure consistent performance. This includes checking for the correct concentration of anti-SRBC antibodies and verifying the lysis reaction in control experiments.

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Procedure of CFT

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Sample Collection and Preparation

Blood Sample

01

Blood samples are collected from infected animals and processed to obtain serum. The serum is tested for the presence of specific antibodies.

Quality Control

02

The serum must be free from contaminants and properly stored to prevent degradation of antibodies. Quality control steps ensure the integrity of the sample.

Dilution

03

The serum is diluted in a series of steps to determine the titer of specific antibodies. This helps in quantifying the immune response.

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Mixing and Incubation

01

Antigen and Serum

The antigen is mixed with the diluted serum in a test tube. This allows the formation of antigen-antibody complexes.

02

Addition of Complement

Complement is added to the mixture at an optimal concentration. The reaction is then incubated at a specific temperature to allow complement fixation.

03

Indicator System

The indicator SRBC are added after incubation. The mixture is further incubated to allow complement-mediated lysis of SRBC if complement is available.

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Result Observation

Hemolysis Check

The test tubes are examined for hemolysis of SRBC. The absence of hemolysis indicates a positive test result, while hemolysis indicates a negative result.

Titration and Interpretation

The titer of specific antibodies is determined by identifying the highest dilution of serum that still shows a positive result. This information is crucial for clinical interpretation.

Quality Control Checks

Control experiments are performed to ensure the reliability of the test. Positive and negative controls are included to validate the results.

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Clinical Applications of CFT

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Infectious Diseases

Bacterial Infections

CFT is used to diagnose infections caused by bacteria such as Brucellosis and Glanders. It helps in confirming the presence of specific antibodies and guiding appropriate treatment.

Viral Infections

In viral infections like Rinderpest and African Horse Sickness，CFT can detect antibodies that are difficult to measure using other methods. It aids in the diagnosis of acute infections and monitoring of immune response.

parasitic infection

For parasitic infection such as Trypanosomiasis and Toxoplasmosis, CFT provides a reliable diagnostic tool. It helps in differentiating between active and latent infections.

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Autoimmune Diseases

Systemic Lupus Erythematosus (SLE)

In SLE, CFT can detect autoantibodies that are characteristic of the disease. It helps in confirming the diagnosis and monitoring disease activity.

Rheumatoid Arthritis (RA)

CFT is used to detect rheumatoid factor (RF) antibodies in RA patients. It provides additional diagnostic information and helps in assessing disease severity.

Other Autoimmune Conditions

CFT is also useful in diagnosing other autoimmune diseases such as scleroderma and Sjögren's syndrome. It helps in identifying specific autoantibodies and guiding treatment strategies.

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Serological Surveillance

Epidemiological Studies

CFT is employed in epidemiological studies to monitor the prevalence of specific infections in animals. It helps in identifying outbreaks and assessing the effectiveness of vaccination programs.

Vaccine Efficacy

In vaccine trials, CFT is used to measure the immune response to vaccination. It helps in determining the efficacy of vaccines and optimizing immunization schedules.

Public Health Interventions

CFT data supports public health interventions by providing insights into the spread of infectious diseases. It aids in planning and implementing control measures to prevent outbreaks.

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Limitations of CFT

Sensitivity and Specificity

While CFT is a valuable diagnostic tool, it has limitations in terms of sensitivity and specificity. False positives and false negatives can occur due to factors such as cross-reactivity and low antibody titers.

Technical Challenges

The procedure of CFT is complex and requires skilled technicians. Variability in antigen preparation, complement activity, and incubation conditions can affect the results.

Time-Consuming

CFT is a time-consuming test, involving multiple steps and incubation periods. This can delay the diagnosis and treatment of animals.

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Limitations and Future Directions

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Future Directions

Advances in Technology

01

Advances in technology, such as automated systems and improved antigen preparation methods, are expected to enhance the accuracy and speed of CFT.

Integration with Other Tests

02

Combining CFT with other diagnostic tests, such as PCR and ELISA, can improve the overall diagnostic accuracy. This integrated approach can provide a more comprehensive assessment of infections and immune responses.

Research and Development

03

Ongoing research aims to develop new antigens and indicators for CFT. This will expand its applications and improve its reliability for diagnosing a wider range of diseases.

Thank you!

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