SEROLOGICAL TEST AND APPLICATIONS

Universiti Malaysia Kelantan
Faculty of Veterinary Medicine

Immunology and Serology (dVT2153)

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SEROLOGICAL TESTS

- Any of several laboratory procedures carried out on a sample of blood serum

- The purpose of such a test is to detect serum antibodies or antibody-like substances that appear specifically in association with certain diseases

- Particularly helpful in the diagnosis of bacterial and viral diseases.
Immunoturbidimetry and Nephelometry

- Measure the turbidity of a sample.

- Antibody-antigen clusters form immune complexes that precipitates and increase turbidity of sample.

- When light is passed through, some light is scattered by the sample, some is absorbed by the sample and the rest passes through the sample.

- Immunoturbidity measures the absorbance of the light by the sample & nephelometry measures the light scattered at a fixed angle.
Principles

Turbidimetry
- Detector at 180°C to incident light.
- Measure the decrease in transmitted light.

Nephelometry
- Angle of detection varies, commonly 90°.
- Measuring the amount (intensity) of the light scattered.
Applications

- To measure canine albumin in urine and cerebrospinal fluid
Enzyme Linked Immunosorbent Assay (ELISA)

- ELISA is an immunoassay technique designed to detect and quantify peptides, proteins, antibodies and hormones.

- Antigen must be immobilized to a solid surface and then bind with an enzyme conjugated antibody.

- ELISAs are performed in 96-well plates and the bottom of each well is coated with a protein to which the antibody binds.
Principles of ELISA

1. Antibody is attached to a solid substrate
   Antigen from specimen are bind to the capture antibody

2. Enzyme labeled antibody (horseradish peroxidase/ alkaline phosphatase) is added
   Organic substrate for the particular enzyme is added

3. The colored product of the reaction of the enzyme on the substrate can be detected visually
   Read by spectrophotometer
Principles of ELISA
Types of ELISA

- **Direct ELISA** uses the method of direct labeling of the antibody itself.

- **Indirect ELISA** utilizes an unlabeled primary antibody in conjunction with a secondary antibody.

- **Sandwich ELISA** is used to detect sample antigens bound between the capture antibody & the detection antibody.
Types of ELISA

Direct Assay

Indirect Assay

Capture Assay “Sandwich”
Applications of ELISA

- Detection of *Mycobacterium* antibodies in tuberculosis case.
- Detection of rotavirus in feces.
- Detection of hepatitis B markers in the serum.
- Detection of enterotoxin of *E.coli* in feces.
- Detection of HIV in blood samples
- Detection of potential food allergens
Agglutination Tests

• Agglutinin: Ab that agglutinate particulate Ag

• All Ab can theoretically agglutinate particulate Ag

• IgM (high valency) is a good agglutinating antibody
Agglutination Tests

**Qualitative**
- To detect presence of an antigen or an antibody
- Ab is mixed with particulate antigen. +ve: agglutination

**Quantitative**
- To measure the level of Ab to particulate Ag
- Serial dilutions
- Fixed no. of RBC/bacteria/Ag added
- Max dilution that gives agglutination (titer)
Slide Agglutination Test
(salmonella- Typhoid fever)
Tube Agglutination Test
(Bruceellosis screening)
Serum Plate Agglutination Test (SPA)
(Salmonella, Mycoplasma)
Immuno Chromatographic Test

- Rapid strip based immunoassays
- The physical principle relies on the migration of antigen or Antigen-Antibody complex along the membrane
Immuno Chromatographic Test
Immuno Chromatographic Test

- A labeled antibody binds to the antigen of interest
- Ag-Ab complex are then immobilized in the support matrix by an unlabeled antibody bound to the matrix
- Results seen as colored spots or bands
Benefits

- Fast result: results can be read in 20 min
- Easy to use: just adding a drop of sample in the right container
- Relatively inexpensive to make
- No special technical requirement
- No lab work required
**Viral Haemagglutination**

- Some viruses and microbes contain proteins which bind to erythrocytes (red blood cells) causing them to clump together.

For diagnosis of:

- NDV
- Adenovirus III
- AIV
- IBV
- Mycoplasma
Titer = 32 HA units/ml

Hemagglutination test method

serial dilution

mix with red blood cells

side view

top view

One HA unit: minimum amount of virus that causes complete agglutination of RBCs
Readings the Results

- **Titer:** maximum dilution that gives visible agglutination.

- **The end point:** is the well with the lowest concentration of the virus where there is haemagglutination

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- The HA titer of this virus in this row is 256 or $2^8$ (1:256 dilution contains (1 HA unit) (one haemagglutinating unit)
Haemagglutination

A
Type A blood of donor + "Anti-B" agglutinins of type A recipient → No agglutination

B
Type B blood of donor + "Anti-B" agglutinins of type A recipient → Agglutination + Hemolysis
Measures compounds in biological fluids precisely and extreme sensitively

Principles

• Uses antibodies to detect and quantitate the amount of antigen (analyte) in a sample

• Based on formation of Ag-Ab complex

• Highly sensitive due to presence of radioactivity that can be detected in tracer quantities
RADIO IMMUNO ASSAYS (RIA)

- **Principle**
  - High specificity due to highly specific immunologic reaction between Ag and Ab
  
  - Basic principle: competitive binding, where a radioactive antigen ("tracer") competes with a non-radioactive antigen for a fixed number of antibody or receptor binding sites
  
  - In case of a 'direct' technique, a radioactively labelled anti-IgG subclass-specific antibody is used. In an 'indirect' technique, an anti-IgG subclass-specific antibody directed against the first antibody

- **Uses**
  - Useful in assay of hormones, enzyme, steroids and peptides in plasma
  - Determination of the serum level of a drug that has been administered to a patient
RADIO IMMUNO ASSAYS (RIA)

Radioactive antigen

“First” antibody

Add unlabeled antigen (○)

Radioactive antigen (○) displaced by unlabeled antigen (○)

Precipitate ag-Ab complexes with anti-immunoglobulin (“second” antibody)

Radioactivity of supernatant = free antigen

Radioactivity of precipitate = bound antigen

“Second” antibody
RADIO IMMUNO ASSAYS (RIA)
Immunofluorescent Antibody Test (IFAT)

- Analogous to RIA except:
  - Label: fluorophore rather than radioisotope

- Categorized under heterogenous or homogeneous assays which can be performed in competitive or non-competitive respectively

- **Principle**
  - Antibody present in the serum will bind to the antigen.
  - This antigen-bound antibody is subsequently detected by the use of a secondary antibody that has been conjugated to a fluorochrome.
  - Deposition of the fluorochrome is observed by use of the fluorescence microscope
Immunofluorescent Antibody Test (IFAT)

- **Uses**
  - Detecting seropositivity to an infectious agent
  - Detects antibodies in serum or other body fluids, most often antibodies specific for an infectious agent or an autoantigen
  - A confirmatory test for FeLV, if the cat tests positive to the ELISA test
Direct FAT

Antibody against the viral antigen labeled with FITC

FITC

Viral antigen

Example: Direct FAT for detecting rabies viral antigen

Indirect FAT

Antibody against viral antigen prepared in rabbit

Antibody against rabbit labeled with FITC

FITC = fluorescein isothiocyanate
Immunofluorescent Antibody Test (IFAT)

CVS-11: Rabies challenge virus standard
MNA: mouse neuroblastoma cell
Complement Fixation Test (CFT)

- Complement fixation referred as the binding and activation of complement to Ag-Ab complex.

- Complement fixation can detect antibody level of less than one microgram per ml.

- It was widely used to diagnose infections, particularly with microbes that are not easily detected by culture methods, and in rheumatic diseases.
Complement Fixation Test (CFT)
Complement Fixation Test (CFT)

- The complement fixation assay can be used to look for the presence of:
  - Specific antibody
  - Specific antigen in a patient's serum.

- The test utilizes sheep red blood cells (SRBC), anti-SRBC antibody and complement, along with specific antigen (for detection of antibody in serum) or specific antibody (for detection of antigen in serum).
Principles of CFT

If antibody (or antigen) is present in the patient's serum
• Then the complement is completely utilized and SRBC lysis is minimal.

If the antibody (or antigen) is not present in the patient's serum
• Then the complement binds anti-SRBC antibody and lysis of the SRBCs ensues.
Principles of CFT

$\text{Ag} + \text{Ab} + \text{complements} \rightarrow \text{Indicator system} \rightarrow \text{NO HEMOLYSIS}$

$\text{SAMPLE} \ (\text{No Ag}) + \text{Ab} + \text{complements} \rightarrow \text{Indicator system} \rightarrow \text{HEMOLYSIS}$
Complement Fixation Test

1. Serum with antibodies
2. Antigen binds with antibodies
3. Complement binds with Ag/Ab complex
4. Hemolysin Sensitized red blood cells serve as an indicator
5. RBCs settle into a pellet
6. No lysis
   - Reactive

7. Serum without antibodies
8. Unbound Antigen
9. Unbound complement
10. Hemolysin Sensitized RBCs serve as an indicator
11. RBCs lysed by unbound complement
12. Lysis
   - Nonreactive
Applications of CFT

- The complement fixation test to *C. burnetii* is used to detect the presence of antibodies to *Coxiella burnetii* bacteria in the blood. These highly infectious bacteria causes Q fever.

- Cytolytic or cytocidal tests also are complements dependent. When a suitable live bacterium such as *Vibrio cholera* is mixed with its antibody in the presence of complement, the bacterium is killed and lysed. This forms the basis of the vibriocidal antibody test for the measurement of anticholera antibodies.

- Complement fixation tests are used routinely for detecting viruses in tissue cultures which have been inoculated with specimens of blood or tissue fluids from humans with probable viral infections.
Applications of CFT

- The classical complement fixation test is the Wassermann reaction used in the diagnosis of syphilis which is caused by *Treponema pallidum*

  - Consists of Wassermann antigen mixed with dilutions of the patients’ serum in the presence of guinea pig complement.

  - After the antigen and patients’ serum have had time to react and take up the limited amount of complement available in the system, the indicator system is added to show whether or not there is a free complement.

  - Controls are included to ensure that none of the reagents are anticomplementary and positive and negative control sera are tested in parallel.

  - This test is also known immobilization test.
PRECIPITATION TEST

- In precipitation antigen combines with its antibody in the presence of electrolytes (Nacl) at a suitable temperature and Ph the antigen and antibody complexes form in soluble precipitate suspended as floccules.

- Reaction can take place in liquid medium, gels, agar, agarose, polyacrylamide.
Mechanisms of Precipitation

- Precipitation occurs with most antigens because the antigen is multivalent (i.e. has several antigenic determinants per molecule to which antibodies can bind).

- Antibodies have at least two antigen binding sites (and in the case of IgM there is a multimeric complex with up to 10 antigen binding sites), thus large aggregates or gel-like lattices of antigen and antibody are formed.
Principle of Precipitation Test

- Soluble antigen + antibody (in proper proportions) → visible precipitate

- Lattice formation (antigen binds with Fab sites of 2 antibodies)

Examples of specific tests:
- Double diffusion (Ouchterlony)
- Single diffusion (radial Immunodiffusion)
- Immunoelectrophoresis
- Immunofixation
Serum Neutralization Test

• The “gold standard“ for Bovine Viral Diarrhoea (BVD) antibody detection. It is suitable for the detection and quantification of BVD-specific antibodies in serum.

• Depending on the test setup, it provides information on:
  • Recent infections
  • Infective status of a herd
  • The efficiency of a vaccination program
Serum Neutralization Test

- A procedure in which the chemical or biological activity of a reagent or a living organism is inhibited, usually by a specific neutralizing antibody.

- Serum + known viral suspension

- If Abs to the virus are present, Abs bind to the virus, preventing its attachment to & subsequent infection of cells

- When virus is then added to an appropriate cell culture, it is unable to replicate & cause cell death

- Also to used to test for toxins
A serum sample is diluted in steps and incubated with the same quantity of virus.

If there are enough antibodies in the serum the viruses are neutralized.

In order to make the neutralization effect visible, susceptible cells are added to the samples.

If neutralization is successful the cells remain intact, i.e. a cytopathic effect is not recognized or an immune staining remains negative.

The SNT is sensitive and specific, compared to the ELISA, it has, however, disadvantages regarding labour and dependence on cell cultures.
Serum Neutralization Test

- Serial dilutions of test sera
- Addition of virus
- First incubation
- Addition of cells
- Second incubation
- Plate reading
Toxin and Antitoxin Test

- Antitoxins are basically antibodies produced in response to antigenic toxins.

- Antitoxins are normally prepared against a toxin such as diphtheria or botulism or against animal venoms such as black widow spider venom or various snake venoms.

- Since toxins are soluble antigens, in vitro interactions with antitoxins are seen as precipitation.

- Properly, toxins are poisonous proteins, especially of bacterial origin. However, nonproteinaceous poisons, such as fungal aflatoxins and plant alkaloids, are also often called toxins.
Toxin and Antitoxin Test

- Flocculation: a process wherein colloids come out of suspension in the form of floc or flakes by the addition of a clarifying agent.

- Clarifying agents- substances used to remove suspended solids from liquids during flocculation.
Toxin and Antitoxin Test

- With horse antitoxin, flocculation occurs only if toxin and antitoxin are near equivalence, a two fold excess of either reactant giving soluble complexes.

- If the antitoxin is derived from any species other than the horse, precipitation occurs over a wide range of reactant ratios, as in other antigen-antibody reactions.

- In most instances, the reaction results in partial or complete neutralization of the toxic activity of the antigen.
Toxin and Antitoxin Test

T5000 - *C. difficile* Toxin/Antitoxin Kit

- A toxin/antitoxin kit for the detection of *Clostridium difficile* toxin in fecal specimens.
- After treatment with antibiotics, many patients develop gastrointestinal problems ranging from mild diarrhea to severe pseudomembranous colitis.
- Many cases of the milder forms of gastrointestinal illness and most cases of pseudomembranous colitis are caused by *Clostridium difficile*. 
Toxin and Antitoxin Test

**T5000 - C. difficile Toxin/Antitoxin Kit**

- The organism is an opportunistic anaerobic bacterium that grows in the intestine once the normal flora has been altered by antibiotics.
- *Clostridium difficile* produces two toxins, A and B.
- Toxin B, often referred to as the cytotoxin, is the toxin detected by the tissue culture assay.
- The *C. difficile Toxin/Antitoxin Kit* uses a tissue culture format to detect the presence of cytotoxic activity (cell rounding) in fecal specimens and confirms the identification of *C. difficile* toxin by using specific antitoxin.
Toxin and Antitoxin Test

T5000 - *C. difficile* Toxin/Antitoxin Kit

- Reagents in the kit include toxin control and specific antitoxin.

- Features:
  - Lyophilized reagents
  - Highly sensitive
  - Minimizes repeated testing of specimens containing high toxin levels