Wondershare EdrawMind

immunoassay

- Precipitation
  - zones
    - prozone phenomenon:antibody excess, no lattice network is formed.
    - postzone phenomenon: antigen excess. no lattice network is formed.
    - zone of equivalence
      - a lattice (line or lattice) formed
      - Ags & Abs at optimal or equivalent proportions
  - Single radial diffusion On a slide
    - steps
      - Ab incorporated in agar gel
      - Ag. added to wells in agar.
      - Ag. diffuses radially from the well
      - Forms precipitation ring around antigen
      - Diameter of halo – estimate of conc. of Ag.
        - proportional
    - Used for estimation of
      - Igs levels in serum,
      - screening Abs against viruses (Influenza) or bacteria
  - doeble immunodiffusion
    - steps
      - diffusion of Ab and Ag towards each other in agarose gel
      - a line will form if there is binding between them
    - Ouchterlony results for
      - the detection of antigens and antibodies
      - determination of homologies between antigen
- agglutination
  - direct
    - hemagglutination.
      - Uses
        - blood grouping
          - Antisera of the IgM type can be used to in blood grouping
          - method
            - Smooth suspension of blood on 3 slides + drop of antibody on each slide
            - . Clumping of blood means it has that antibody specific antigen
        - cross matching
    - identification and typing of micro-organisms as
      - pneumococci
      - Brucella agglutination test
      - widal test
        - detects typhoid fever
        - The antigens used include Salmonella O (somatic) and H (flagellar) antigens.
    - titer test
      - serial dilutions are made of a antibody sample (patient serum) and then a fixed number of particulate antigen is added
      - . Then the last dilution that gives agglutination is determined and called the titer
    - coombs test
      - Coombs reagents is anti-human IgG
        - is made in animals or by means of hybridoma techniques
      - direct
        - used to demonstrate in vivo attachment of antibody to an individual's red blood cells.
        - method
          - Patient RBC sample is mixed with coombs reagent
          - if agglutination occurs this mean that the RBCs have antibody on their surfaces (sensitized)
        - detects
          - autoimmune hemolytic anemia
          - hemolytic disease of the newborn
          - sensitization of red blood cells caused by the presence of drugs, or a transfusion reaction.
      - indirect
        - used to determine the presence of a particular antibody in a patient.
        - method
          - Patient serum is mixed with RBC have the RH antigen, and the cells are then carefully washed again to remove any unbound antibody.
          - coombs reagent is added, a visible reaction occurs if the patient is positive for the antibody
        - uses
          - screen pregnant women for antibodies that may cause hemolytic disease of the newborn ,
            - Pregnant serum + RH+ RBC
          - screen for antibodies in the preparation of blood for blood transfusion (Cross match).
            - Recipient serum + donor RBC
  - passive
    - latex test
      - Ab is attached to the carrier and Ag is detected
        - CRP,RA factor,HCG, hepatitis B
      - Ag is attached to the carrier and Ab is detected
        - meningococci, influenza type B
    - Coagglutination
      - Staphylococcus aureus is most frequently used,
        - because it has a protein on its outer surface, called protein A, which naturally adsorbs the (FC) portion of antibody molecules.
    - Passive heamagglutination
      - known antigen is coated on RBC and then mixed with serum from patient
      - TAPH test, treponema pallidum heamagglutination test for syphilis
    - reverse PAT
      - Ab linked to latex
        - e.g lancefield grouping strepto
  - agglutination inhibition test
    - method
      - patient's serum is added to reagent Ab
      - is Ag is present in serum, it binds Ab

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- Immuno-cyto/histo-chemistry
  - detect the presence and localization of antigens in tissues (histo-) or in cells (cyto-) grown in culture
  - uses radiolabeled antibody and Immunofluorescent microscope
  - uses
    - in identification of anti-nuclear antibody and autoantibodies in autoimmune diseases
    - cancer
  - Visualizing an antibody-antigen interaction can be accomplished by
    - Immunofluorescence
      - Chromogenic immunohistochemistry (CIH),
        - an antibody is conjugated to an enzyme, such as peroxidase
      - Indirect Immunofluorescence
        - the antibody is tagged to a fluorophore, such as fluorescein
        - Uses in tumor antigen detection, ANA in SLE and Rheumatoid arthritis and in autoimmune diseases.
        - Direct Immunofluorescence ,
          - attaching fluorescein to a specific mouse antibody directed against the antigen
        - fluorescein can be attached to a second anti-antibody* that is used to bind to the first unlabeled antibody.
          - *e.g., rabbit anti-mouse Ig antibody
        - is more sensitive than direct immunofluorescence since there is amplification of the signal
        - Test ANA
          - patterns
            - peripherla(rim)
              - Anti-DNA Ab
              - SLE
            - homogenous(diffue)
              - Anti-DNA Ab
              - RA and SLE
            - speckled
              - Anri-Sm and RNP
              - SLE and SE
            - centromere
              - anti-centromere An
              - PSS(CREST)
            - nucleolar
              - anti-nuclear Ab
              - SLE and PSS
      - is not an ideal tool to identify the detailed structure of the cell or tissue because of a low structural details.
      - confocal microscopy
        - Antibody can be coupled to an electron-dense probe such as colloidal gold
        - the location of antibody can be determined subcellularly by means of an electron microscope
- complement fixation test
  - steps
    - serum(? Ab)+known Ag+ C, incubate for one hour
    - add indicator system(sheep RBCs anti-sheep RBCs(amboceptor)
    - if serum contains Ab, complement is fixed and there is no haemolysis
  - uses
    - syphilis (Wassermann reaction)
    - Gonorrhea
    - Reckettsial infection
    - Viral infection
  - can use titer test as a quantitative measure
  - anti-complementary reaction
    - when NH in all test tube, RBC and serum control tube
    - due to
      - precense of Ab complex that bind complement and fix it
      - heparin therapy that inactivates complement due to consumption of Ca and Mg
      - old or contaminated serum has destructive action on complement