Immunologic Laboratory Tests
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Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
- IAT
- Immunofluorescence
- ELISA
- Western blot
- Flow cytometry
Things to Remember About Each Test

- What does it measure?
- Where does the Ag-Ab interaction occur?
- How is the Ag-Ab complex detected?
Immunologic Lab Tests Outline

- Agglutination reactions
Agglutination Reactions: Purpose

- Detection of Ag in the patient’s specimen
- Examples:
  - testing for antibodies to infectious agents
  - testing for Hemophilus influenzae type B capsular antigen in CSF
**LATEX AGGLUTINATION**

SPECIMEN FROM A PATIENT WITH ANALYTE OF INTEREST

SPECIMEN FROM A PATIENT WITH ANALYTE OF INTEREST ABSENT

APPLICATION OF SPECIMENS

ANALYTE OF INTEREST

LATEX PARTICLES WITH ANTIBODIES AGAINST THE ANALYTE OF INTEREST (LATEX REAGENT) ARE ADDED

LATEX REAGENT

PATIENT SPECIMEN AND LATEX REAGENT ARE MIXED

AGGLUTINATION

AGGLUTINATION ABSENT

REACTIONS ARE READ IMMEDIATELY

POSITIVE NEGATIVE
negative

positive
Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
DAT: Purpose

- Detection of Ab on patient’s red cells
- Also called the direct Coombs Test
- Performed in patients with hemolytic anemia
patient red cells + AHG = agglutination
Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
- IAT
IAT: Purpose

- Detection of antibodies to red cell antigens
- Also called the indirect Coombs Test
- Performed as part of pre-transfusion testing
patient Ab + donor RBC = Ab-coated donor RBC + AHG = agglutination
Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
- IAT
- Immunofluorescence
Immunofluorescence: Purpose

- Detection of a specific antigen or antibody in a patient’s specimen
- Examples:
  - detection of antigen in a patient’s biopsy specimen
  - detection of antibody in a patient’s serum
IMMUNOF LUORESCENCE

DIRECT
FLUORESCENT LABELED ANTIBODY AGAINST THE ANTIGEN OF INTEREST

SPECIMEN APPLICATION

INCUBATION AND WASH

ANTI-lgG FLUORESCENT LABELED ANTIBODY

INCUBATION AND WASH

FLUORESCENT MICROSCOPE

INDIRECT
ANTIBODY AGAINST THE ANTIGEN OF INTEREST
Positive fluorescent antinuclear antibody test
Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
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- Immunofluorescence
- ELISA
Detection of antibodies in patient specimen

Examples:
• home pregnancy tests
• HIV tests
• tests for some coagulation factors, cytokines, and autoantibodies
ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

1. Patient's serum containing antibodies
2. Ligand coated well
3. Enzyme conjugated anti-human antibodies
4. Enzymatic substrate
5. Detection of enzymatic activity
Immunologic Lab Tests Outline

- Agglutination reactions
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- Western blot
Western Blot: Purpose

- Detection of antibodies in patient specimen
- Most common example: HIV test
PREPARATION OF A REAGENT STRIP

1. Protein suspension
2. Separation by electrophoresis
3. Blotting into nitrocellulose paper
4. Separated proteins and glycoproteins
REAGENT STRIP

PATIENT'S SERUM CONTAINING ANTIBODIES TO PROTEINS ON THE REAGENT STRIP

ANTI-HUMAN IMMUNOGLOBULIN CONJUGATED WITH ALKALINE PHOSPHATASE

BANDS

A B C D E F G H I J K L M
Enough bands = patient is “positive”
Immunologic Lab Tests Outline

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Flow Cytometry: Purpose

- Characterization of cell size, complexity, antigens
- Examples:
  - diagnosis of leukemia and lymphoma
  - determination of CD4/CD8 counts in patients with HIV
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