Immunologic Laboratory Tests
Kristine Krafts, M.D.
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
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
IMMUNOFLUORESCENCE

DIRECT
FLUORESCENT LABELED ANTI BODY AGAINST THE ANTIGEN OF INTEREST

SPECIMEN APPLICATION

INCUBATION AND WASH

ANTI-IgG 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
- IAT
- Immunofluorescence
- ELISA
ELISA: Purpose

• 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

Result: Detection of antibodies
Immunologic Lab Tests Outline

- Agglutination reactions
- DAT
- IAT
- Immunofluorescence
- ELISA
- 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
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

- Agglutination reactions
- DAT
- IAT
- Immunofluorescence
- ELISA
- Western blot
- Flow cytometry
Flow Cytometry: Purpose

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

- Agglutination reactions
- DAT
- IAT
- Immunofluorescence
- ELISA
- Western blot
- Flow cytometry