Procedures for Identifying Pathogens and Diagnosing Infection

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Lec (6)

Clinical Microbiology

Methods of identifying unknown microbes fall into three categories:

1. **Phenotypic** – observable microscopic and macroscopic characteristics
2. **Genotypic** – genetic make up
3. **Immunological** – serology; antibody-antigen reactions

Phenotypic Methods

- Microscopic morphology – fresh or stained microorganisms from specimen; shape, size, stain reaction, cell structures
- Macroscopic morphology – colony appearance; texture, size, shape, pigment, growth requirements
- Physiological/biochemical characteristics – detection of presence or absence of particular enzymes or metabolic pathways
- Chemical analysis – analyze specific chemical composition; cell wall peptides, cell membrane lipids

Genotypic Methods

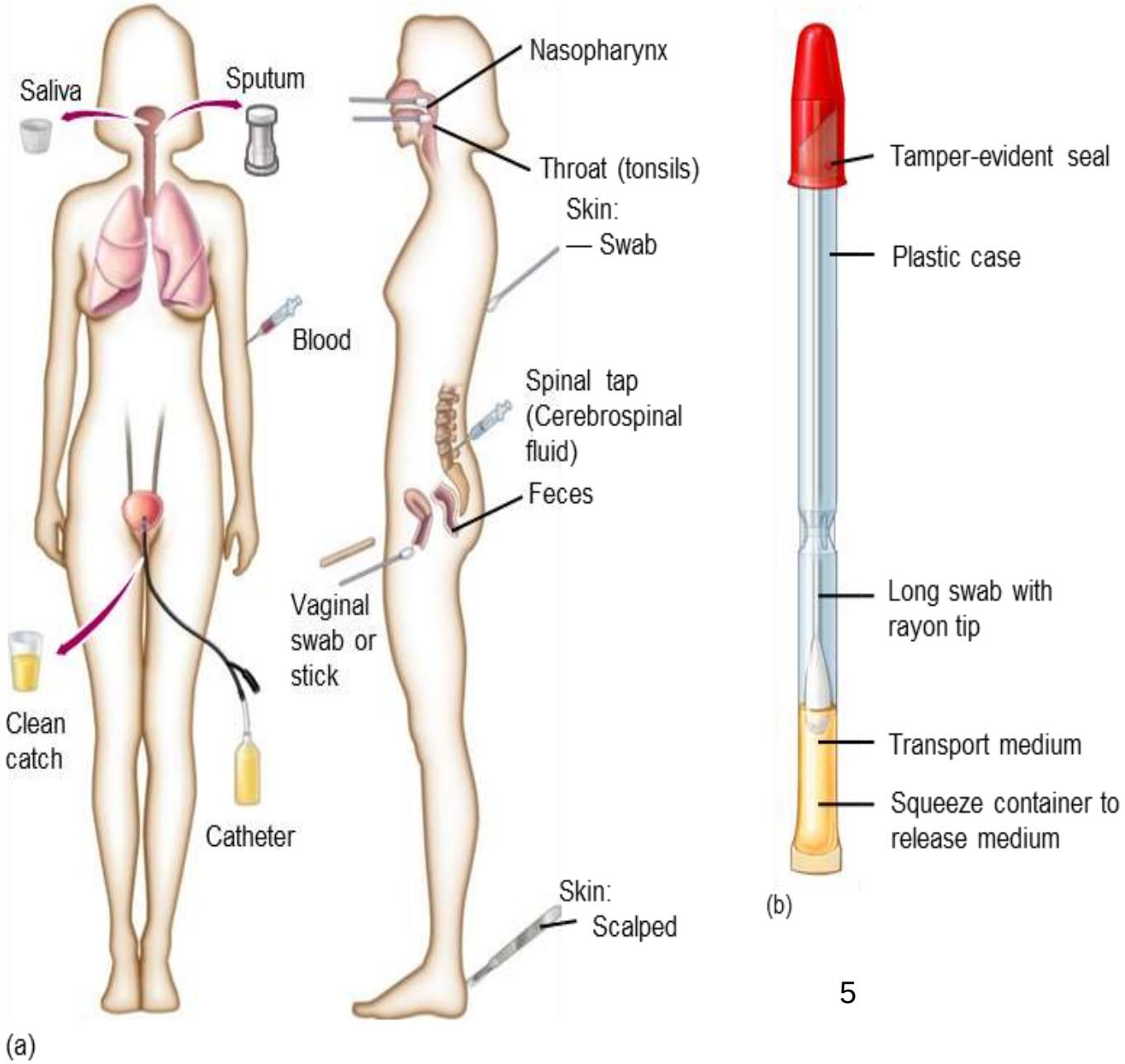
- Assess genetic make-up
- Culture is not necessary
- Precise, automated methods, quick results

Immunological Methods

- Specific antibodies are used to detect antigens
  - Easier than testing for the microbe itself

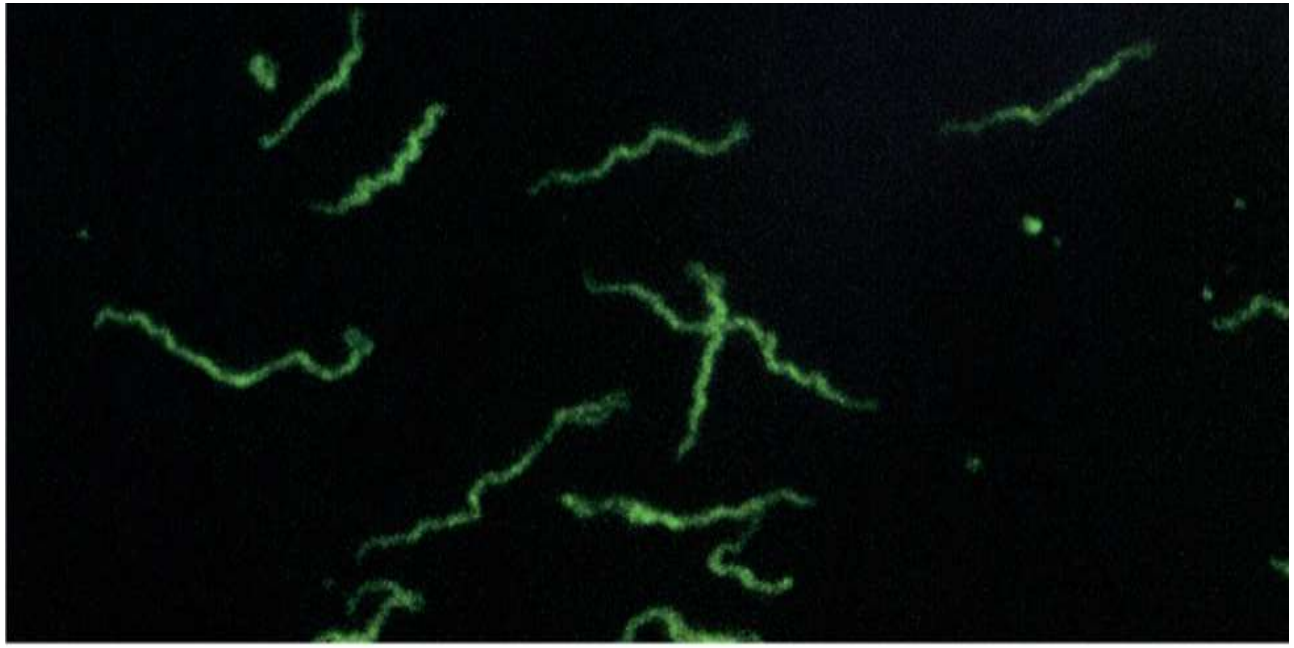
Specimen Collection

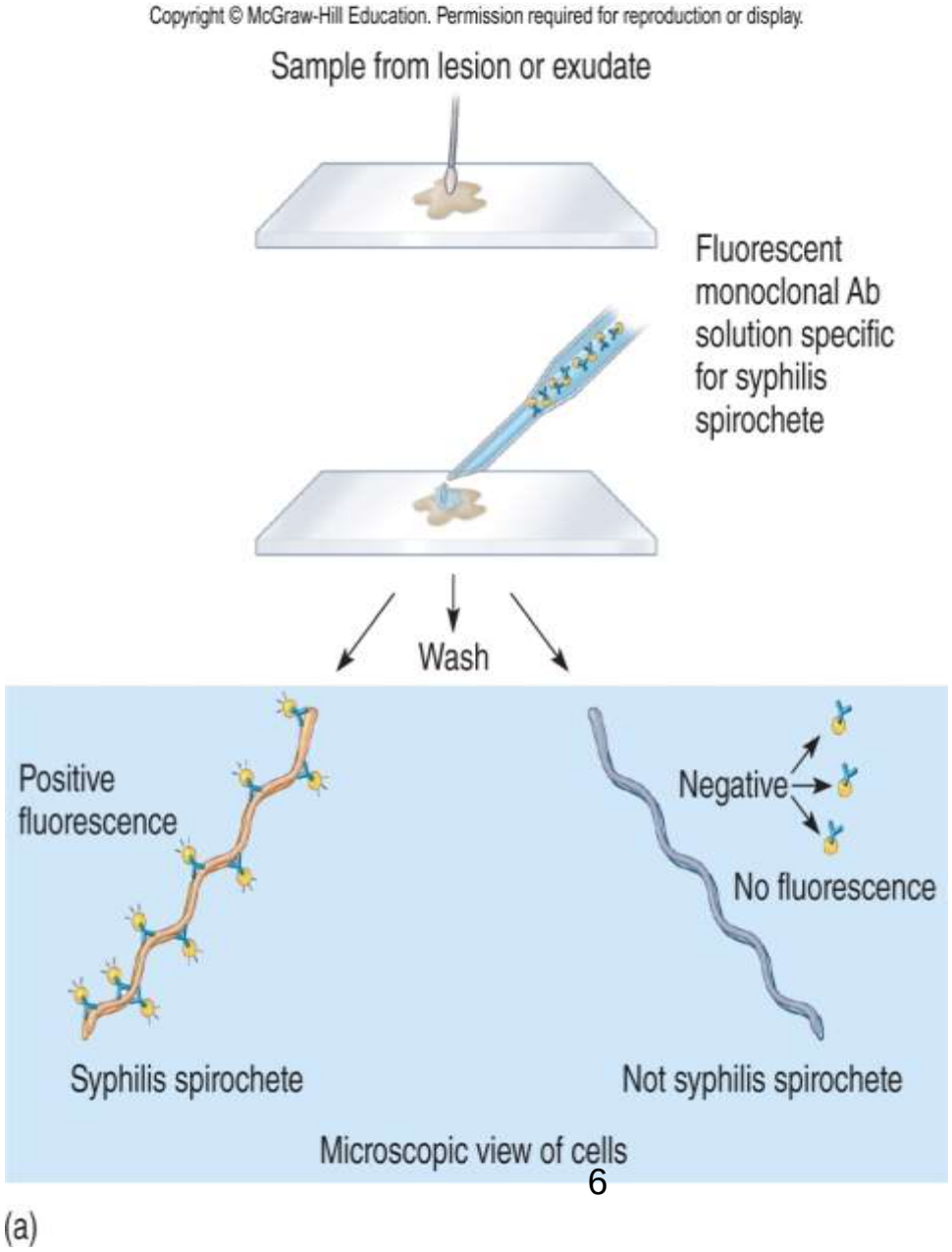
- Sampling body sites or fluids for suspected infectious agent
- Results depend on specimen collection, handling, transport, and storage
- Aseptic procedures should be used



Phenotypic Methods

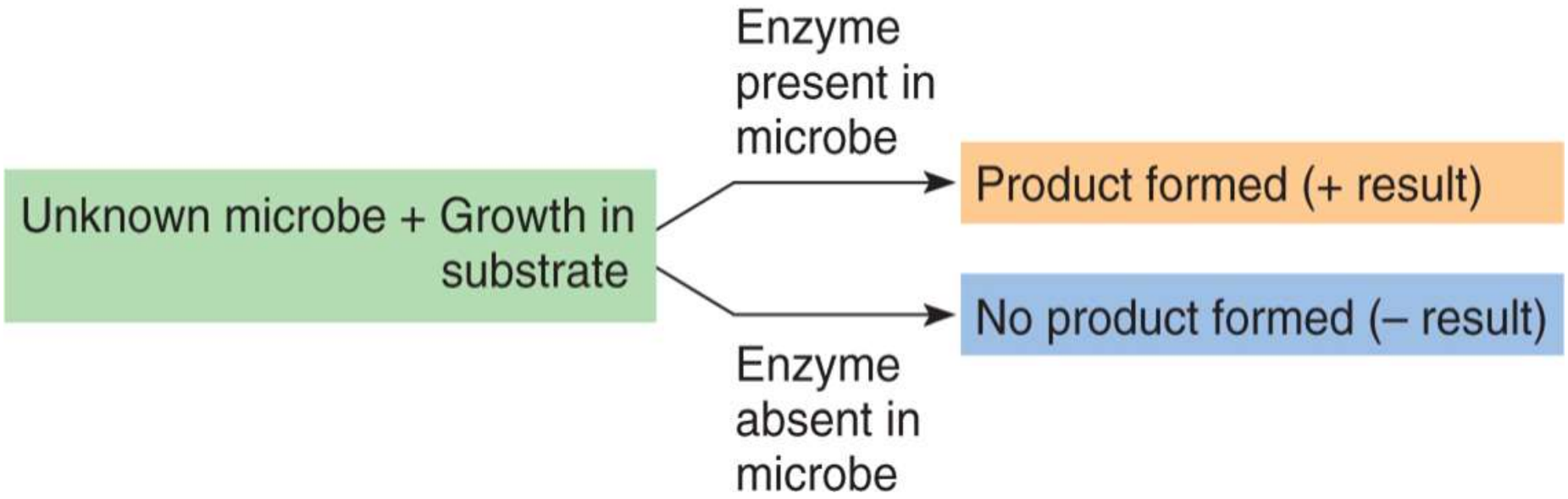
- Immediate direct examination
  - Microscopic – differential and special stains – Gram, DFA (direct fluorescent antibody), direct antigen testing



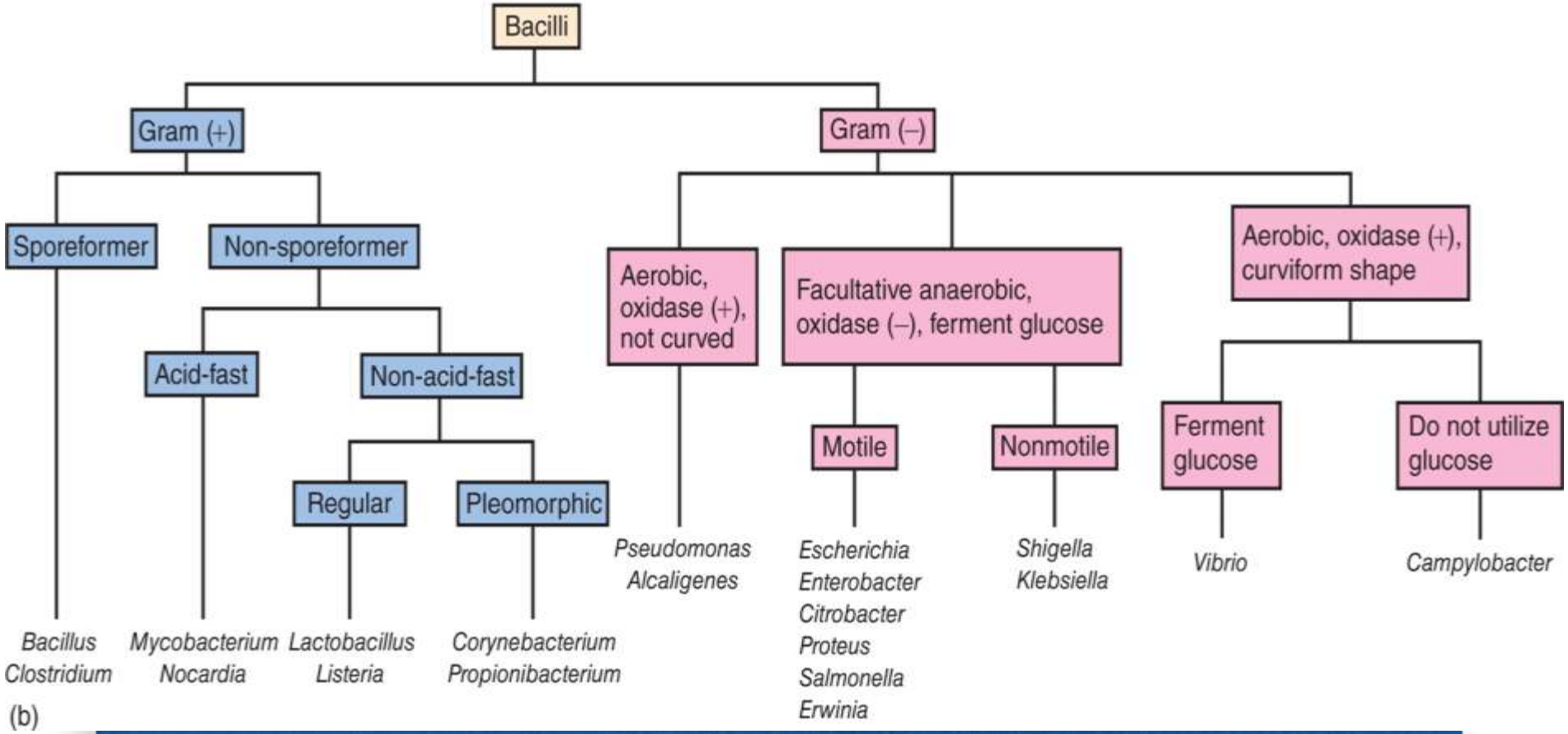


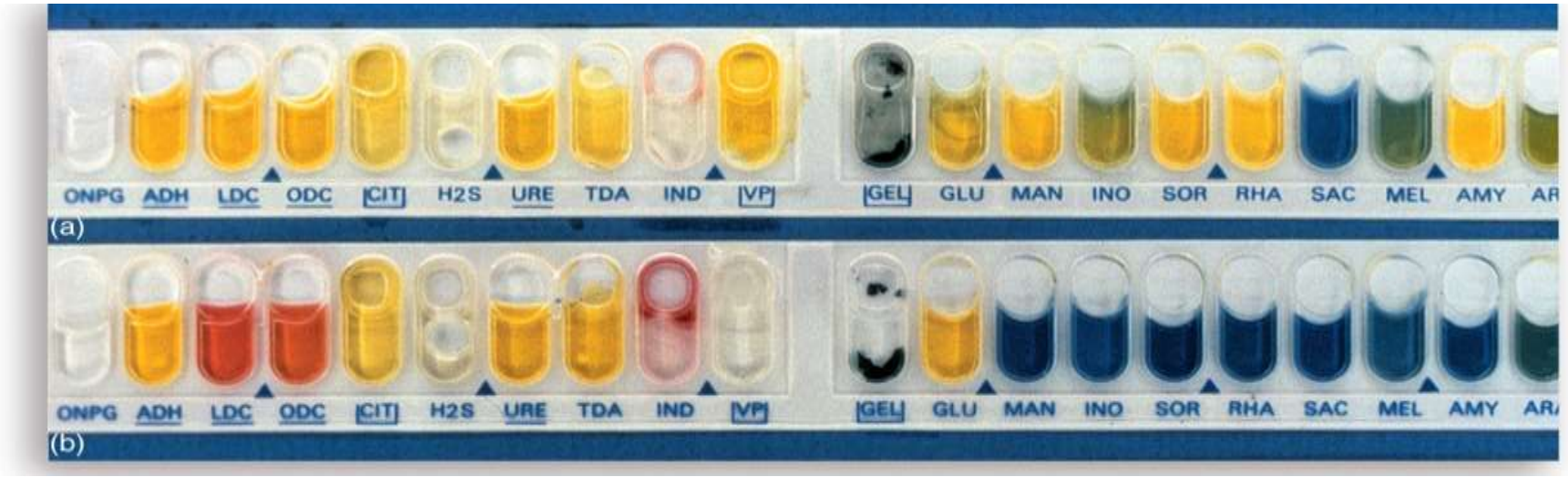
Phenotypic Methods

- Cultivation of Specimen
  - Colony appearance, growth requirements, appropriate media
- Biochemical testing
  - Physiological reactions to nutrients as evidence of the absence or presence of enzymes



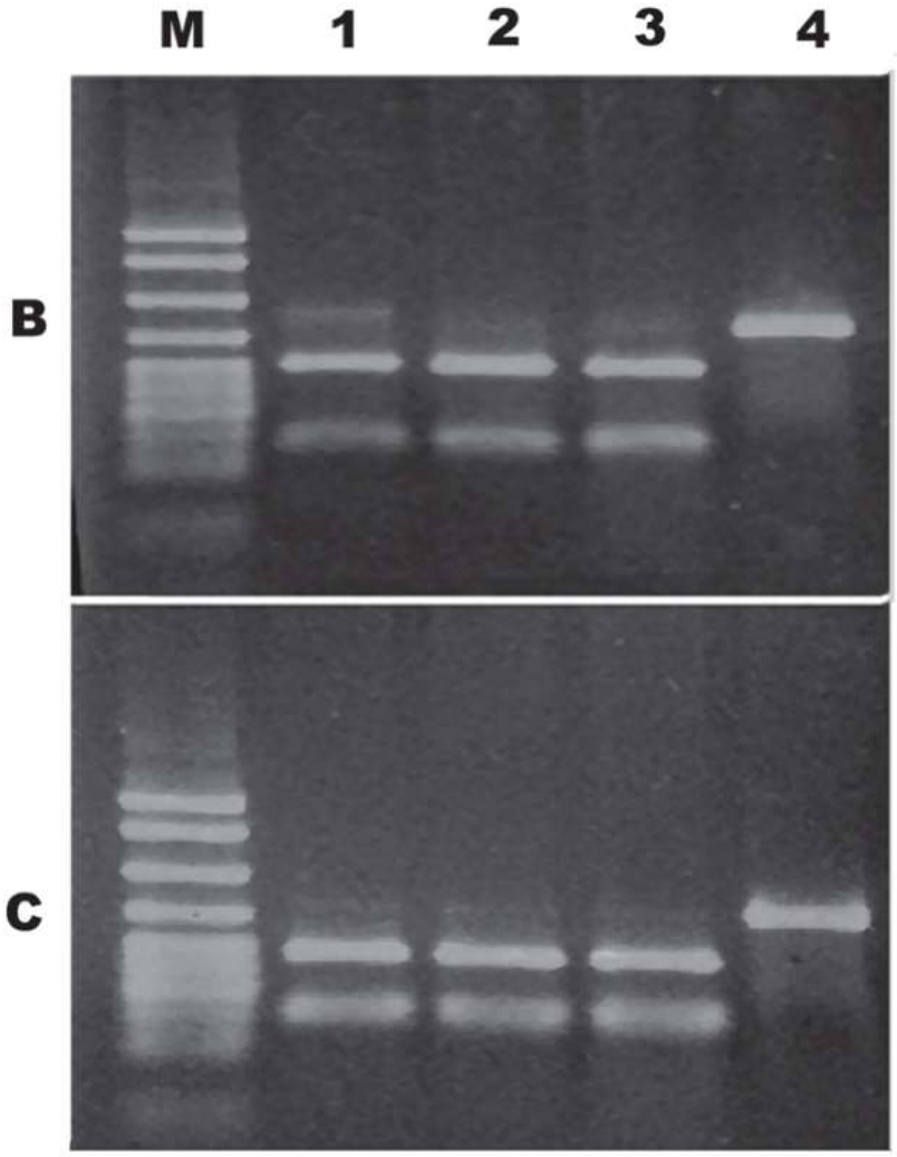
Rapid Tests and Identification





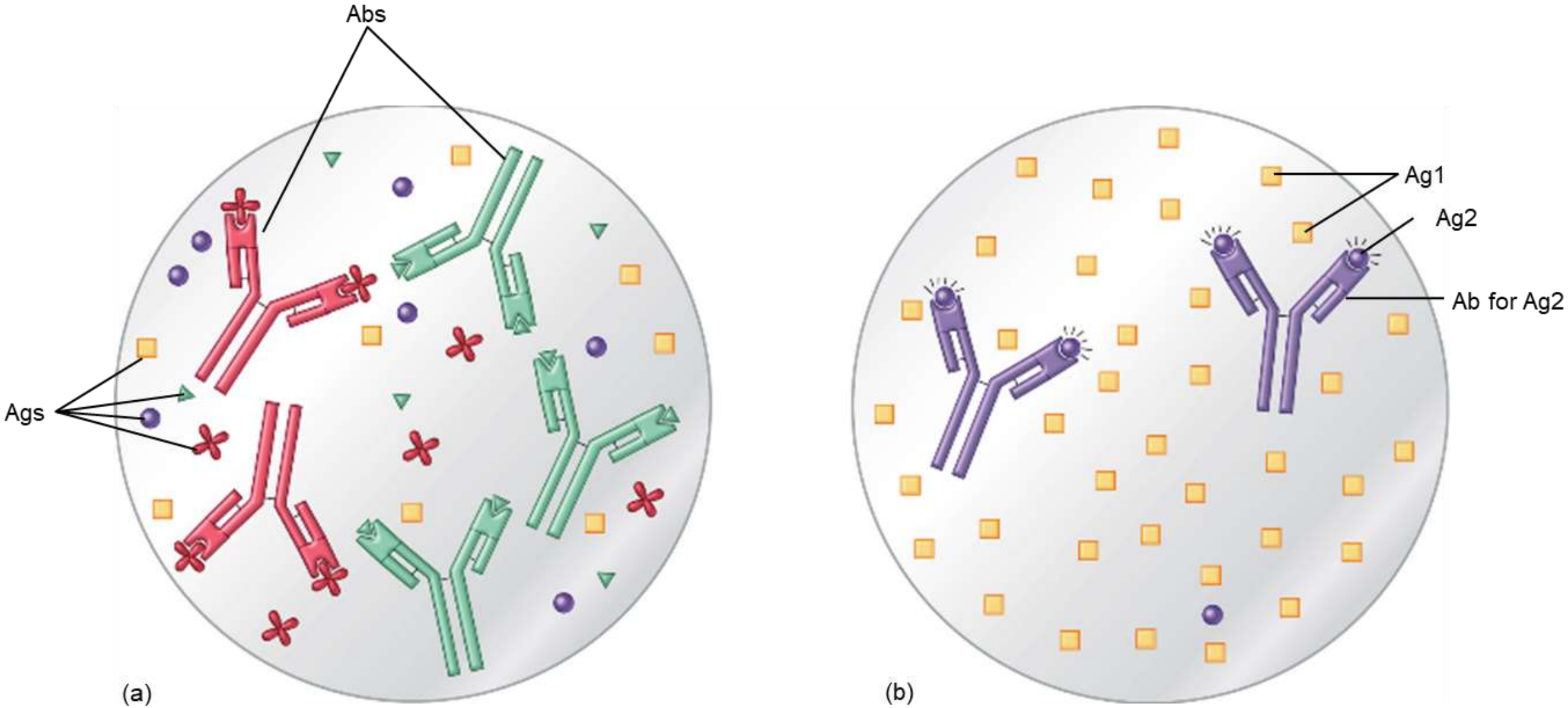
Genotypic Methods

- DNA analysis
  - **Hybridization**
    - Probes complementary to the specific sequences of a particular microbe
  - **PCR**
    - DNA and RNA analysis
    - Ribosomal RNA
      - Comparison of the sequence of nitrogen bases in ribosomal RNA

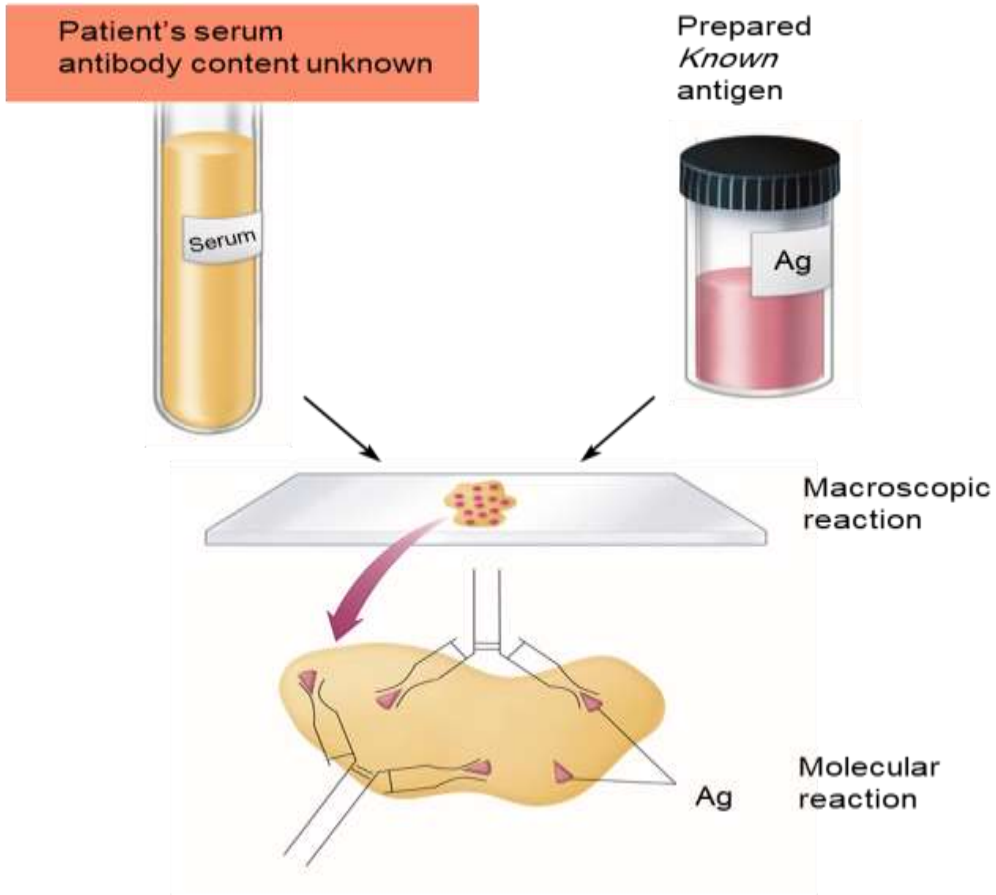


Immunological Methods

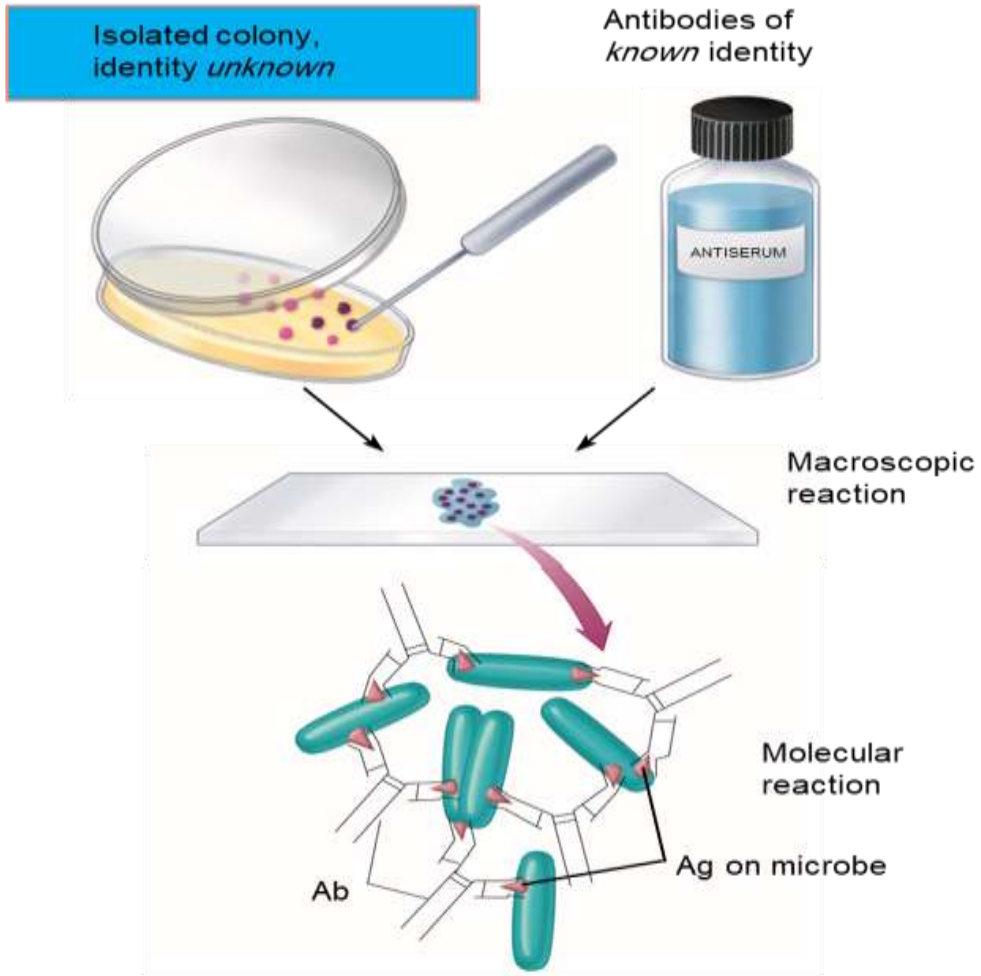
- **Serology** – *in vitro* diagnostic testing of serum
  - Antibodies have extreme specificity for antigens
- Visible reactions include precipitates, color changes, or the release of radioactivity
- Tests can be used to identify and to determine the amount of antibody in serum – **titer**


Abs
Ags
(a)
Ag1
Ag2
Ab for Ag2
(b)

Serological Testing



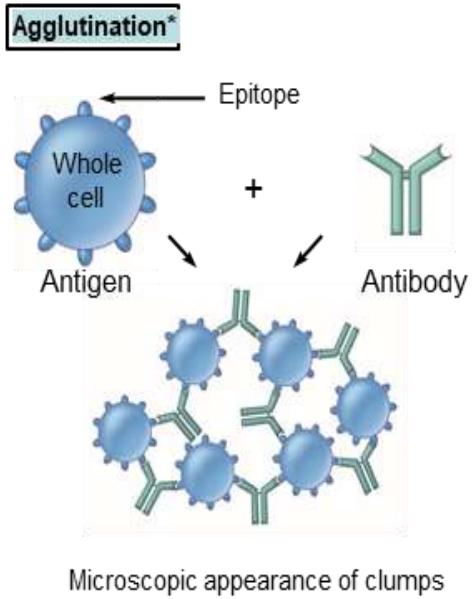
(a) In serological diagnosis of disease, a blood sample is scanned for the presence of antibody using an antigen of known specificity. A positive reaction is usually evident as some visible sign, such as color change or clumping, that indicates a specific interaction between antibody and antigen. (The reaction at the molecular level is rarely observed.)



(b) An unknown microbe is mixed with serum containing antibodies of known specificity, a procedure known as serotyping. Microscopically or macroscopically observable reactions indicate a correct match between antibody and antigen and permit identification of the microbe.

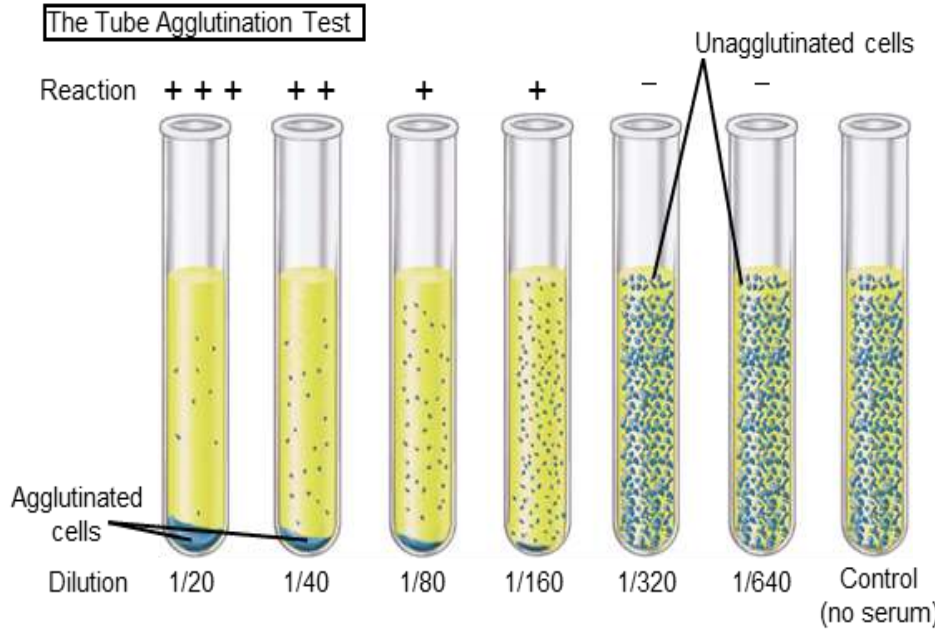
Immune Testing

- **Agglutination testing** – antibody cross links whole-cell antigens, forming complexes that settle out and form visible clumps
  - Blood typing, some bacterial and viral diseases



(a) Agglutination involves clumping of whole cells; precipitation is the formation of antigen-antibody complexes in cell free solution. Both reactions can be observed by noticeable clumps or precipitates in test tubes (see (b) and figure 17.10a).

*Although IgG is shown as the Ab, IgM is also involved in these reactions.

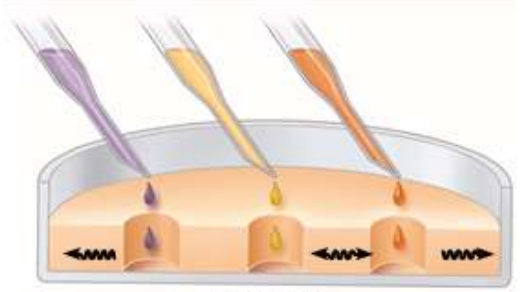


(b) The tube agglutination test. A sample of patient's serum is serially diluted with saline. The dilution is made in a way that halves the number of antibodies in each subsequent tube. An equal amount of the antigen (here, blue bacterial cells) is added to each tube. The control tube has antigen, but no serum. After incubation and centrifugation, each tube is examined for agglutination clumps as compared with the control, which will be cloudy and clump-free. The titer is equivalent to the denominator of the dilution of the last tube in the series that shows agglutination.

Immune Testing

- **Precipitation tests** – soluble antigen is made insoluble by an antibody
  - VDRL(venereal disease research laboratory) ex:syphilis
  - Most precipitation reactions are done in agar gels

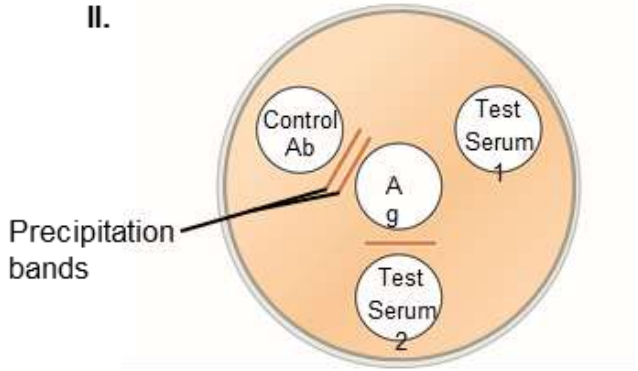
I.



Side view

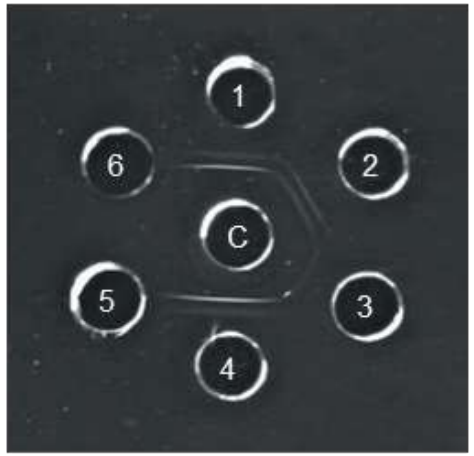
I. In one method of setting up a double-diffusion test, wells are punctured in soft agar, and antibodies (Ab) and antigens (Ag) are added in a pattern. As the contents of the wells diffuse toward each other, a number of reactions can result, depending on whether antibodies meet and precipitate antigens.

II.



II. Example of test pattern and results. Antigen (Ag) is placed in the center well and antibody (Ab) samples are placed in outer wells. The control contains known Abs to the test Ag. Note bands that form where Ab/Ag meet. The other wells (1, 2) contain unknown test sera. One is positive and the other is negative. Double bands indicate more than one antigen and antibody that can react.

III.

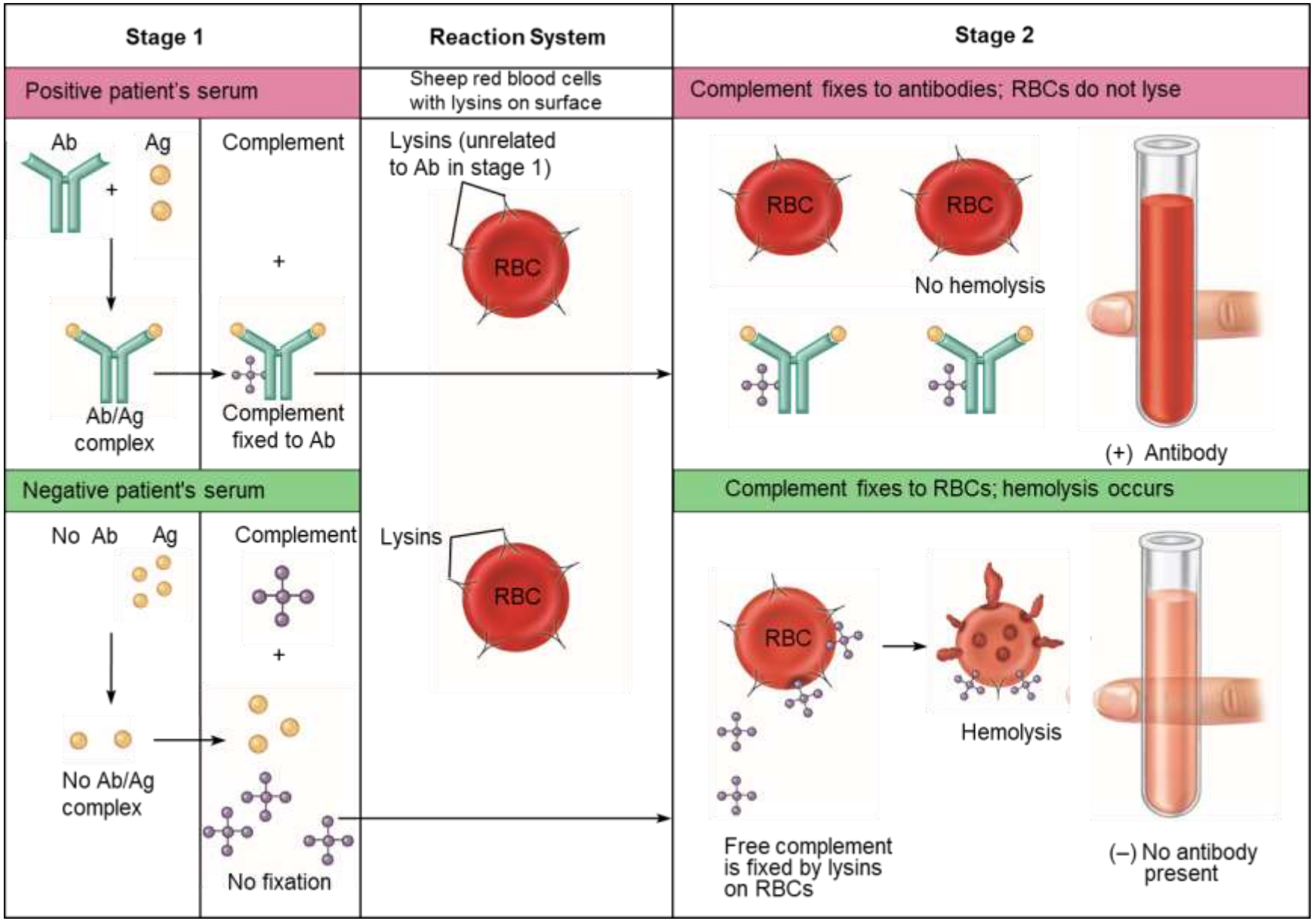


(b)

III. Actual test results for detecting infection with the fungal pathogen *Histoplasma*. Numbers 1 and 4 are controls and 2, 3, 5, and 6 are patient test sera. Can you determine which patients have the infection and which do not?

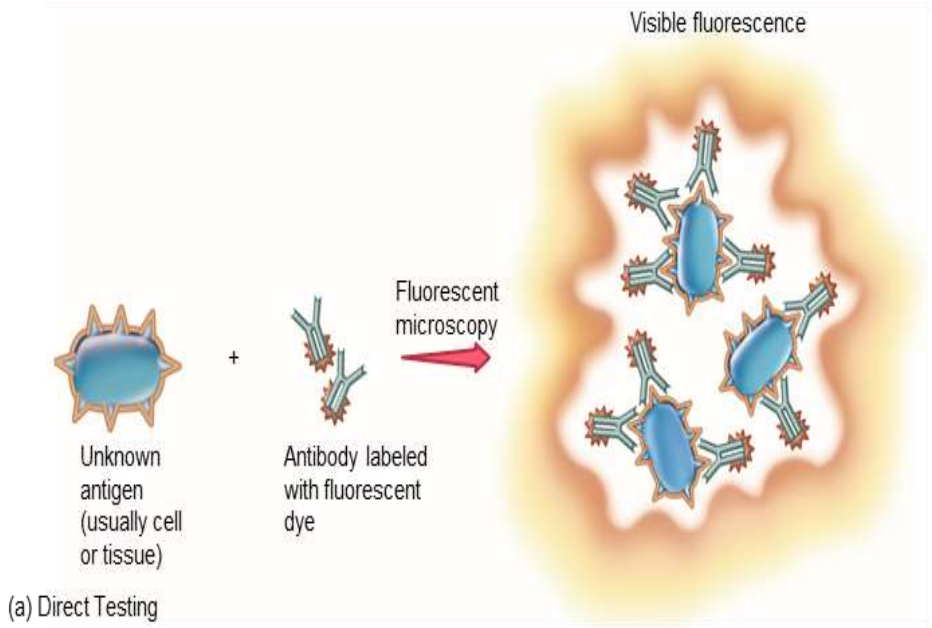
Complement Fixation

- Lysin mediated hemolysis
- Steps of test
  1. Test antigen reacts with test antibody
  2. Contents of tube from (1.) are mixed with sheep RBCs
     - Complement used up in first stage, no hemolysis
     - Unfixed complement, hemolysis

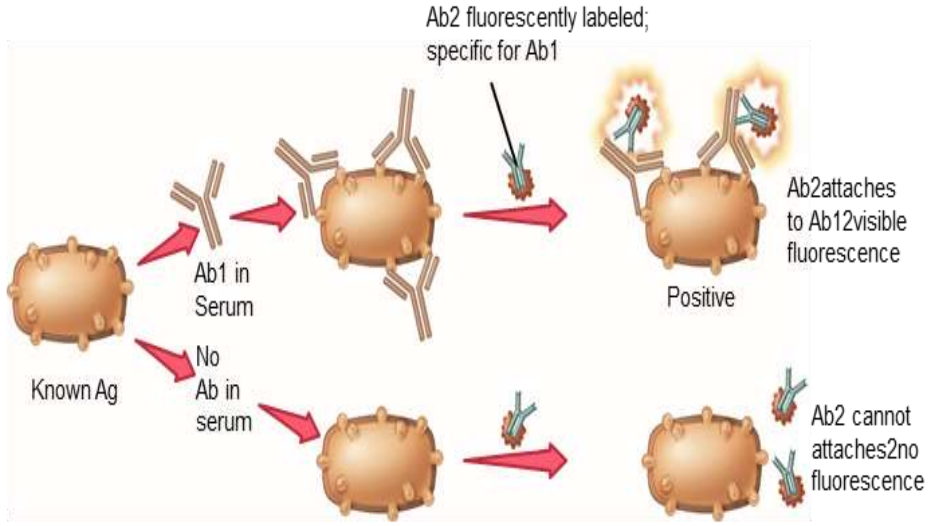

Stage 1
Reaction System
Stage 2
Positive patient's serum
Sheep red blood cells with lysins on surface
Complement fixes to antibodies; RBCs do not lyse
Ab
Ag
Complement
Lysins (unrelated to Ab in stage 1)
RBC
RBC
RBC
No hemolysis
Ab/Ag complex
Complement fixed to Ab
(+) Antibody
Negative patient's serum
Complement fixes to RBCs; hemolysis occurs
No Ab
Ag
Complement
Lysins
RBC
RBC
Hemolysis
No Ab/Ag complex
No fixation
Free complement is fixed by lysins on RBCs
(–) No antibody present

Fluorescent Antibody and Immunofluorescent Testing

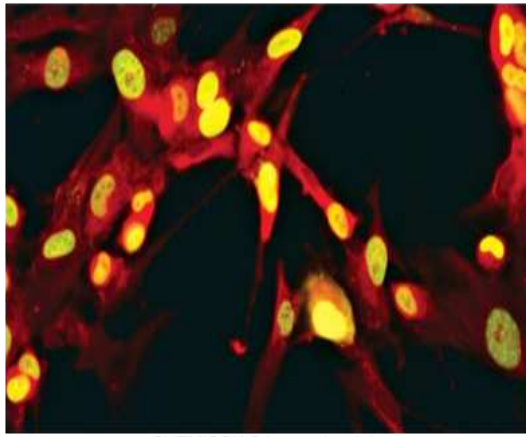
- Fluorescent antibody
  - Monoclonal antibody labeled by a fluorescent dye
- FABs can be used for direct or indirect testing



(a) Direct Testing



(b) Indirect Testing


CHEMICON ® International, Inc.

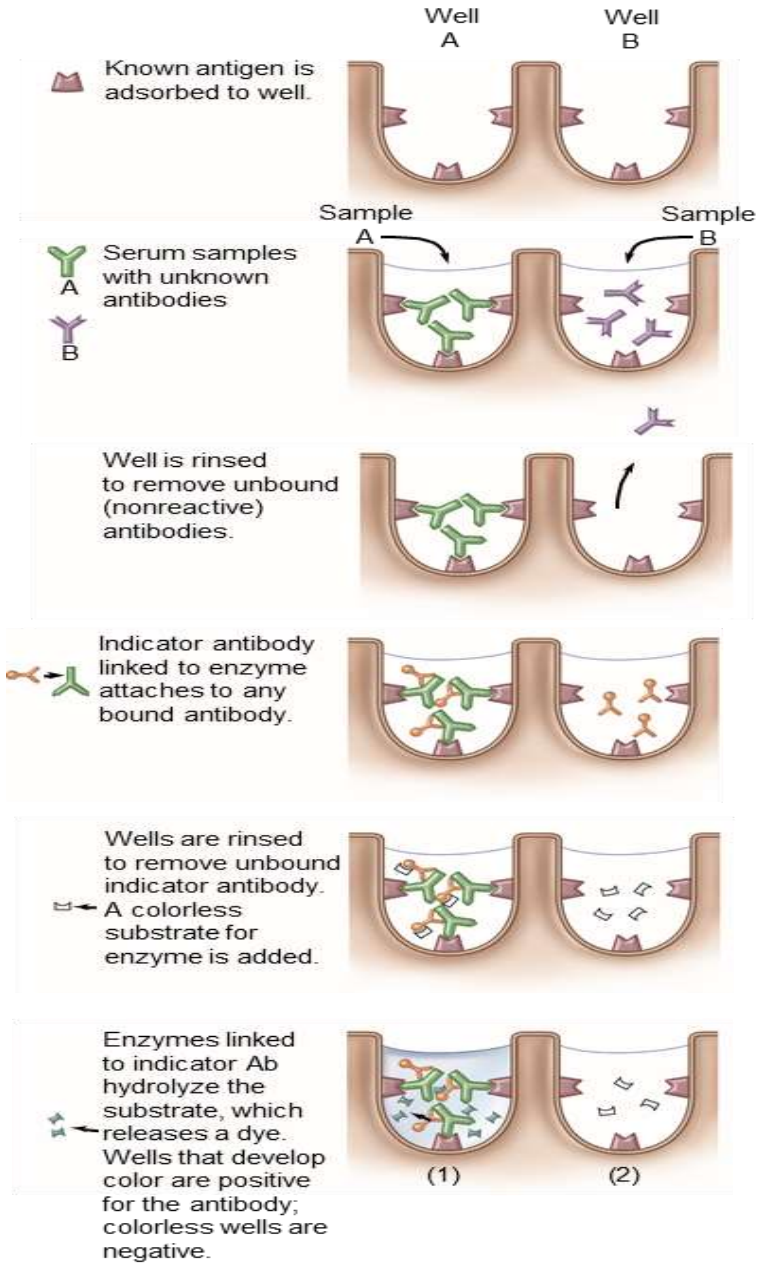
(c) Indirect Immunofluorescence of specimen

Immunoassays

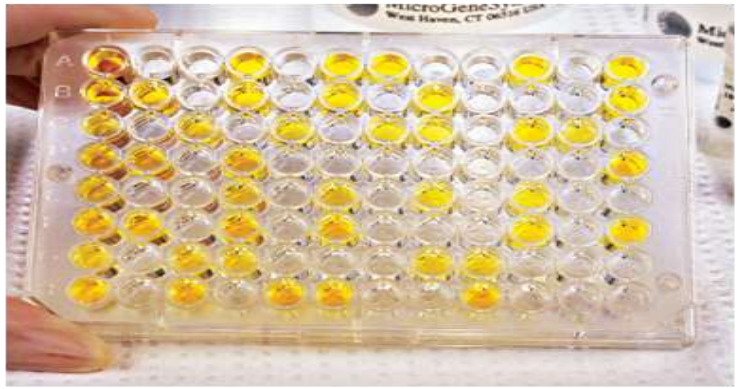
- Extremely sensitive to detect trace antigens and antibodies
- **Radioimmunoassay** (RIA) – antigens or antibodies labeled with radioactive isotopes
- **Enzyme-linked immunosorbent assay** (ELISA) – enzyme-antibody complex produces a colored product when **an** enzyme-substrate reaction occurs
  - Indirect
  - Capture

ELISA Methods

(a) **Indirect ELISA**, comparing a positive vs. negative reaction. This is the basis for HIV screening tests.



(b) **Microtiter ELISA Plate with 96 Tests for HIV Antibodies.** Colored wells indicate a positive reaction.



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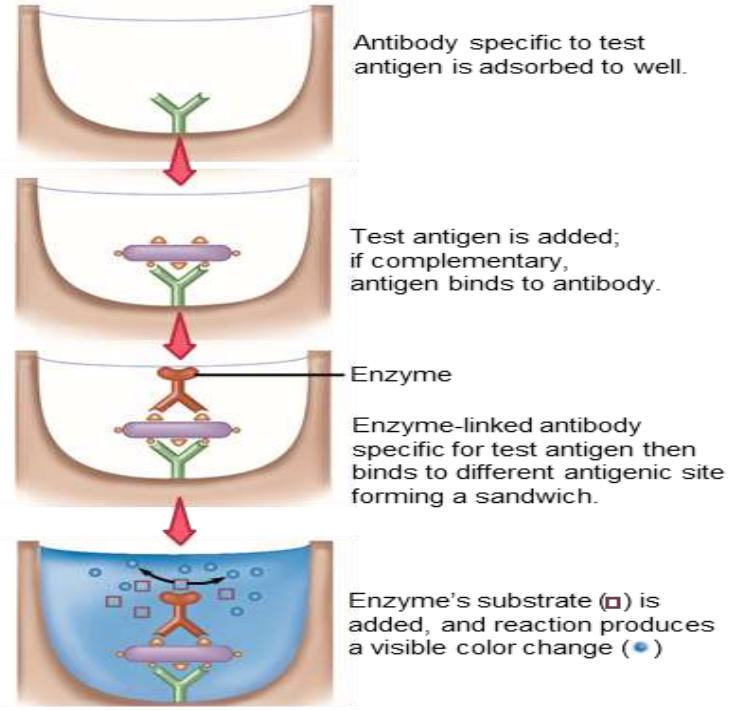
(c) **Capture or Antibody Sandwich ELISA Method.** Note that an antigen is trapped between two antibodies. This test is used to detect the measles virus.



In vivo Testing

- Antigens are introduced directly into the body to determine the presence or absence of antibodies
  - Tuberculin skin test, allergy testing

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