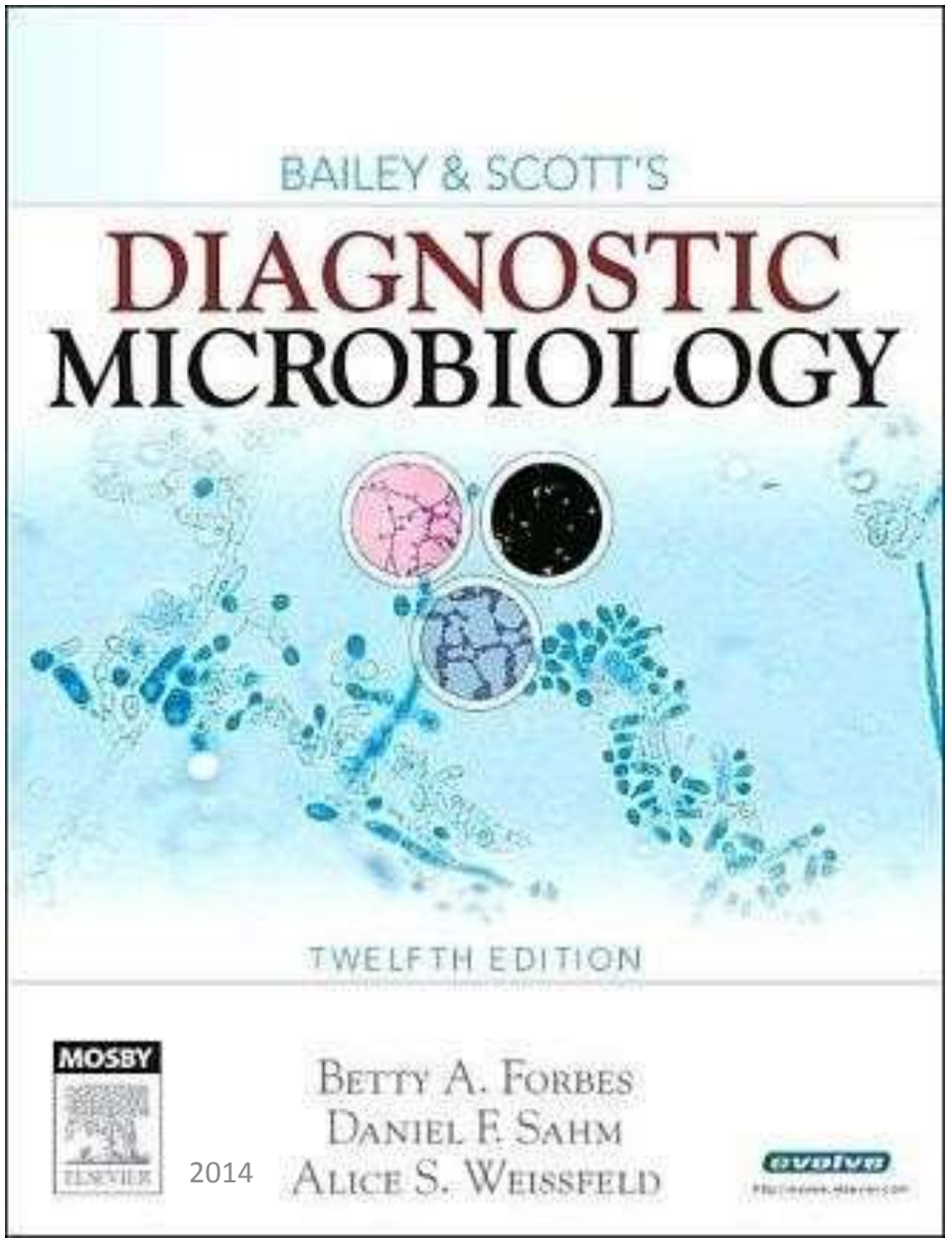
King Saud University
Dept. of Bot. & Microbiology



Microbial Diagnosis

320 MIC

Lecture 8 :

Sample Collection

Sample Collection

•No contamination
•Appropriate equipment
•Good instructions to patient

Collecting the correct specimen

Collecting the Sample Quickly

Take an mid-stream urine (avoids contamination with flora)

CSF (Avoid bloody tap)- (Avoid contamination) **Throat Swab**
Blood cultures (avoid contamination with skin organisms)

• Universal Blood and Body Fluid Precautions

• Stringent measures to prevent the spread of nosocomial infections from patient to patient, from patient to worker, and from worker to patient
•Based on the assumption that all patient specimens could harbor infectious agents, so must be treated with the same degree of care.

Transport to Laboratory

- Safe packaging
- Good labeling
- Temperature

- **Getting the specimen to the lab**
- **Problems in delay or inappropriate storage**

delay in diagnosis & treatment
  - pathogens die
  - contaminants overgrow

- **Blood cultures directly into incubator**
  - not refrigerator

- **CSF straight to lab**
- Don't put an entire surgical specimen into formalin!
  - Send a portion to microbiology in a sterile contaier

Inoculation of Media

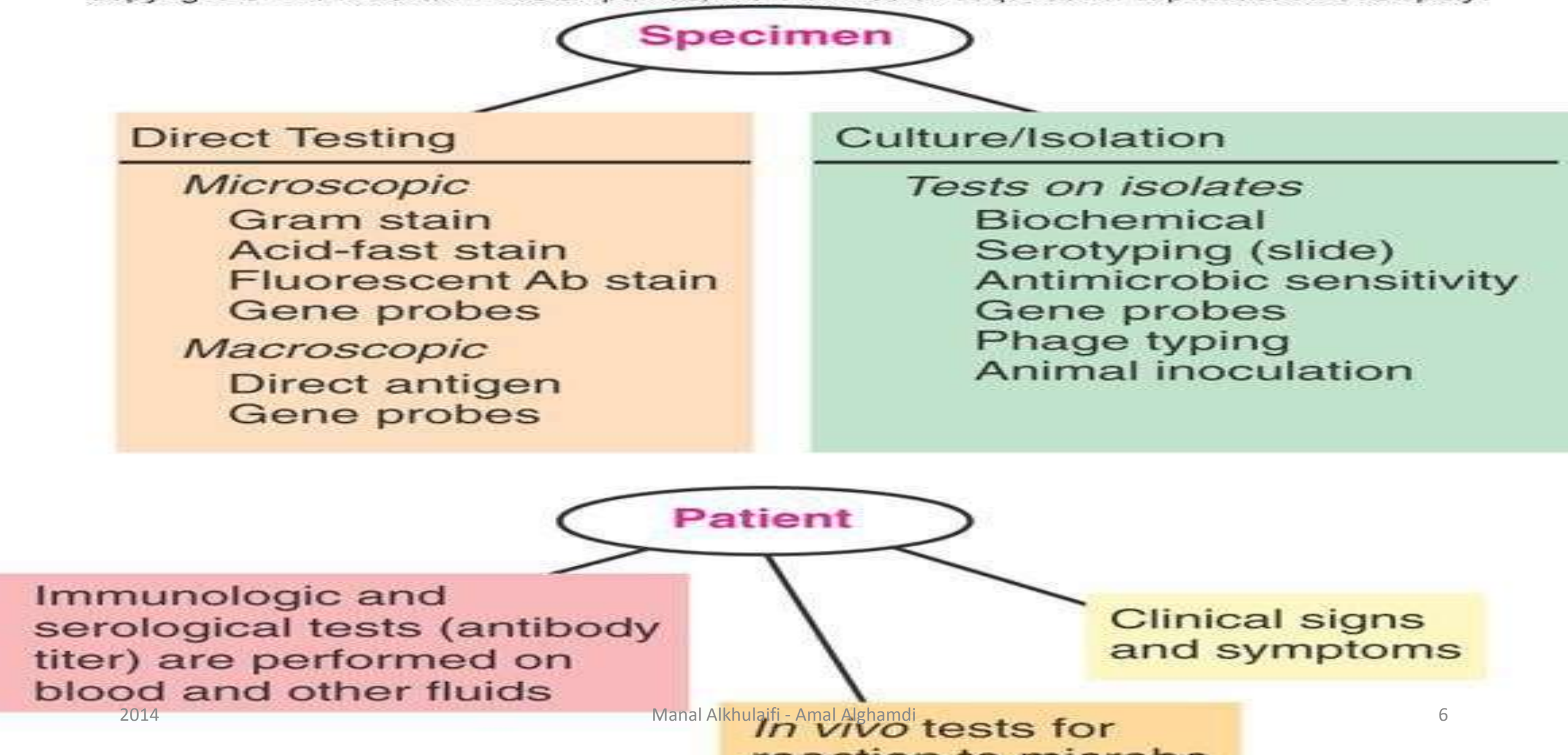
- Use appropriate culture media
  - What kind of specimen is it?
  - What test did the physician request?
- **Culture media**
- Used to grow bacteria or fungus
- **Can be used to:**
  - Enrich the numbers of bacteria or fungi
  - Select for certain bacteria and suppress others – Differentiate among different kinds of bacteria
-
- **The 5 I's of Culturing Microbes**
- **Inoculation –introduction of a sample into a container of media to produce a culture of observable growth**
- **Incubation –under conditions that allow growth**
- **Isolation –separating one species from another**
- **Inspection**
- **Identification**

Documentation

- Specimen is logged in upon arrival in laboratory
- All tests and results are recorded and initialed by microbiologist
- All media and reagents are batch tested with positive and negative controls
- All equipment is checked at least once a day to be sure it is operating within predetermined parameters
    - **Direct and Indirect Testing**

- **Direct:** Demonstration of the presence of an infectious agent
  - Culture
  - Microscopy
  - Molecular methods such as PCR
- **Indirect:** Demonstration of presence of antibodies to a particular infectious agent
  - Serology

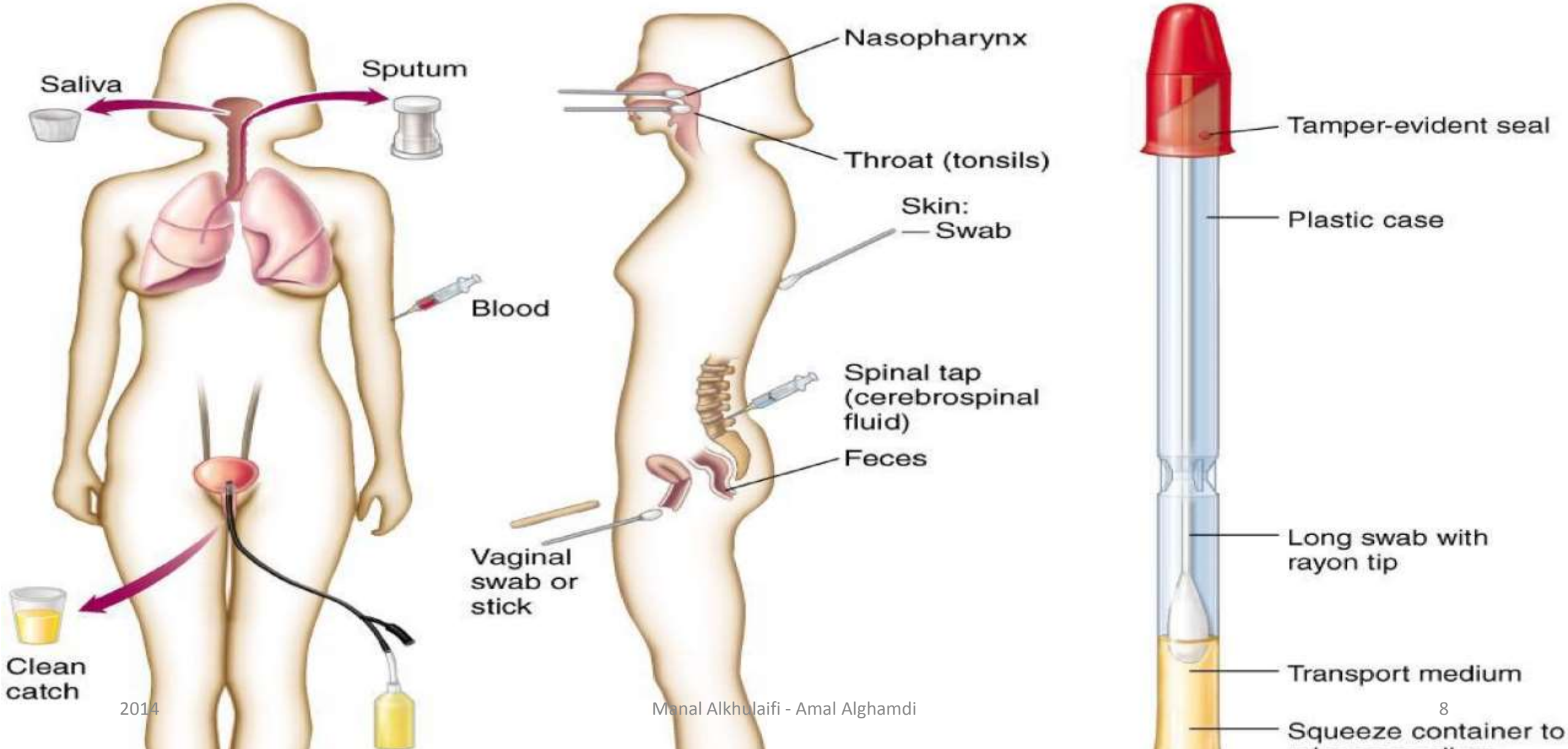
Scheme of specimen isolation and identification



Sterile versus Non-sterile Body Sites

- **Sterile body sites:** These sites normally do not contain any bacteria, so any bacteria found there are significant
- Blood
- Spinal fluid
    -
- **Non-sterile body sites:** These sites are open to the external environment and normally contain bacteria
- Throat
- Feces
- **Specimens from Sterile Sites:** Any organism growing in a normally sterile site is significant
- Identify it
- **Specimens from Non-Sterile Sites:** Only look for specific pathogens
- Physician will order test for a specific organism, or group of organisms
- Other “normal flora” bacteria will be present, but are not be identified.

Samples collection (sampling sites)



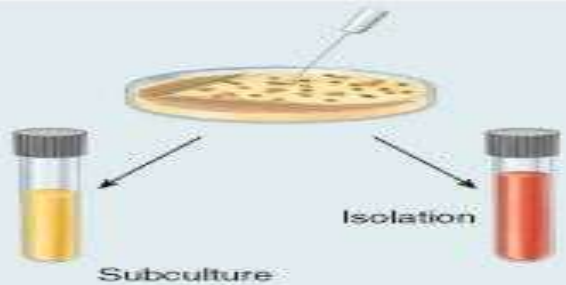
An Overview of Major Techniques Performed by Microbiologists to Locate, Grow, Observe, and Characterize Microorganisms

Specimen Collection:
Nearly any object or material can serve as a source of microbes. Common ones are body fluids and tissues, foods, water, or soil. Specimens are removed by some form of sampling device: a swab, syringe, or a special transport system that holds, maintains, and preserves the microbes in the sample. Discussed on page 58.

A GUIDE TO THE FIVE I's: How the Sample Is Processed and Profiled

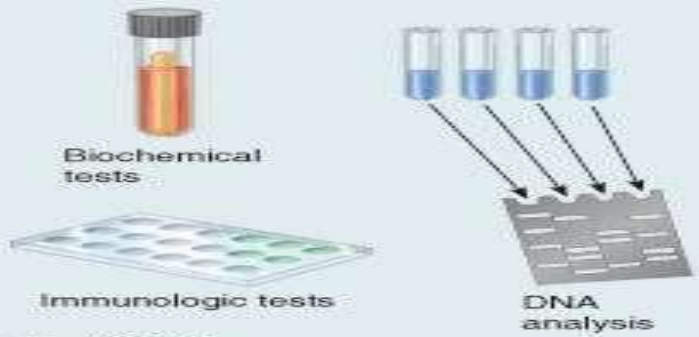
1 Inoculation:
The sample is placed into a container of sterile **medium** containing appropriate nutrients to sustain growth. Inoculation involves spreading a sample on the surface of a solid medium or introducing a sample into a flask or tube. Selection of media with specialized functions can improve later steps of isolation and identification. Some microbes may require a live organism (animal, egg) as the growth medium. Further discussion on pages 60–67.

2 Incubation:
An incubator creates the proper temperature and other conditions conducive to growth. This promotes multiplication of the microbes and usually takes a period of hours or days. Incubation gives rise to a culture—the visible growth of the microbe in or on the medium. Further discussion on page 68.



3 Isolation:
One result of inoculation and incubation is **isolation** of the microbe. Isolated microbes may take the form of separate colonies on solid media, or turbidity (cloudiness) in broths. Further isolation (subculturing) involves taking a bit of growth from an isolated colony and inoculating a separate medium. This makes a pure culture—one that contains only a single species of microbe. More detail on pages 60, 61, 69.

4 Inspection:
The colonies or broth cultures are observed macroscopically for growth characteristics (color, texture, size) that could be useful in analyzing the specimen contents. Slides are made to assess microscopic details such as cell shape, size, and motility. Staining techniques may be used to gather specific information on microscopic morphology. See pages 68, 69.



5 Identification:
A major purpose of the Five "I"s is to determine the type of microbe, usually to the level of species. Information used in identification can include relevant data already taken during initial inspection and additional tests that further describe and differentiate the microbes. These include biochemical tests to determine metabolic activities specific to the microbe, immunologic tests, and genetic analysis. See pages 69, 70.

Requirement for an Infectious Dose (ID)

- Minimum number of microbes required for infection to proceed
- Microbes with small IDs have greater virulence
- Lack of ID will not result in infection

Estimated Infectious Doses of Selected Pathogens*

Agent of	Infectious Dose Estimate	Primary Route of Infection
Measles	1 virus	Respiratory
Q fever	1–10 cells	Respiratory
Tularemia	10–50 cells	Various
Smallpox	10–100 viruses	Respiratory
Brucellosis	10–100 cells	Various
Viral encephalitis	10–100 viruses	Mosquito bite
Plague	100–500 cells	Flea bite
Gonorrhea	1,000 cells	Sexual contact
Anthrax	8,000–50,000 spores	Respiratory, cutaneous
Typhoid	10,000 cells	Ingestion
Cholera	100,000,000 cells	Ingestion

Occurrence of infections with regard to location and sequence

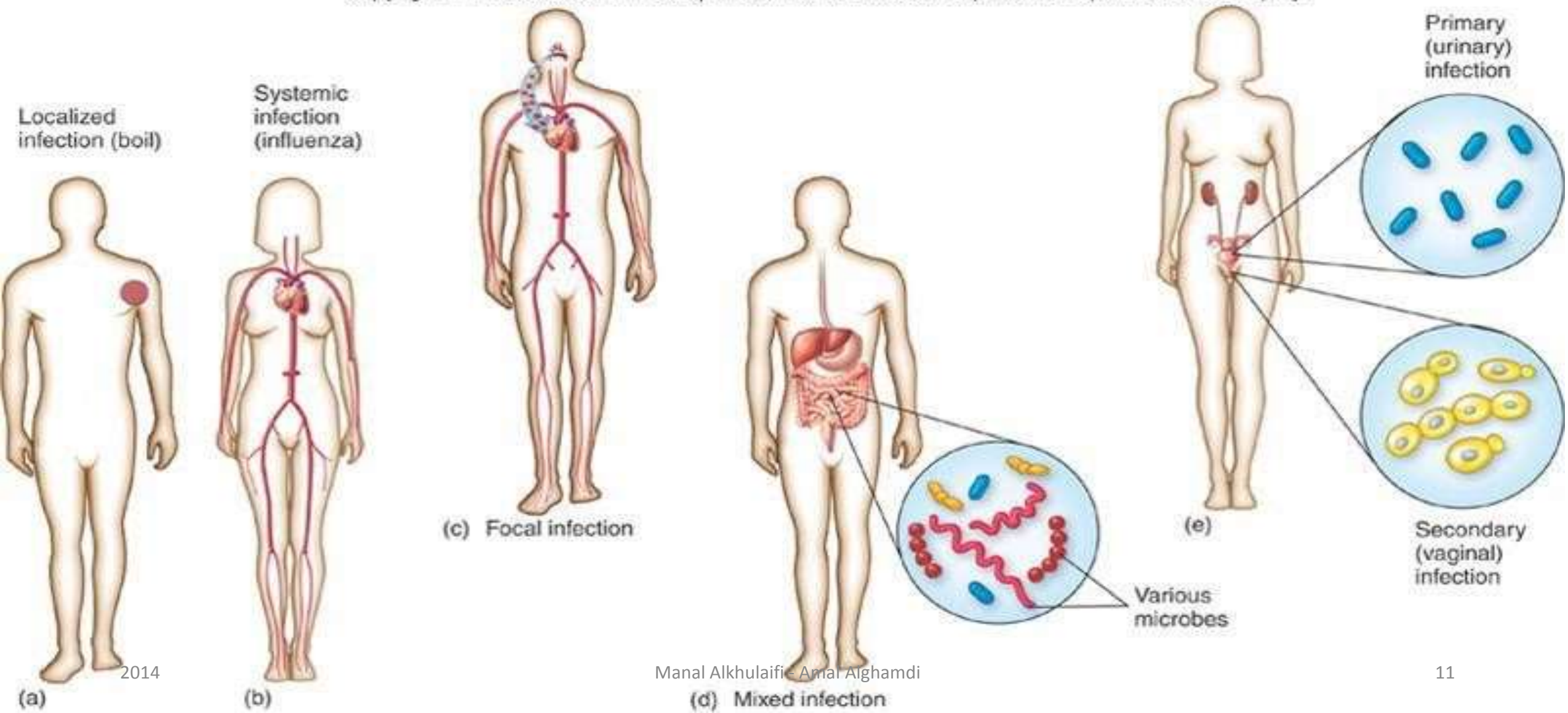
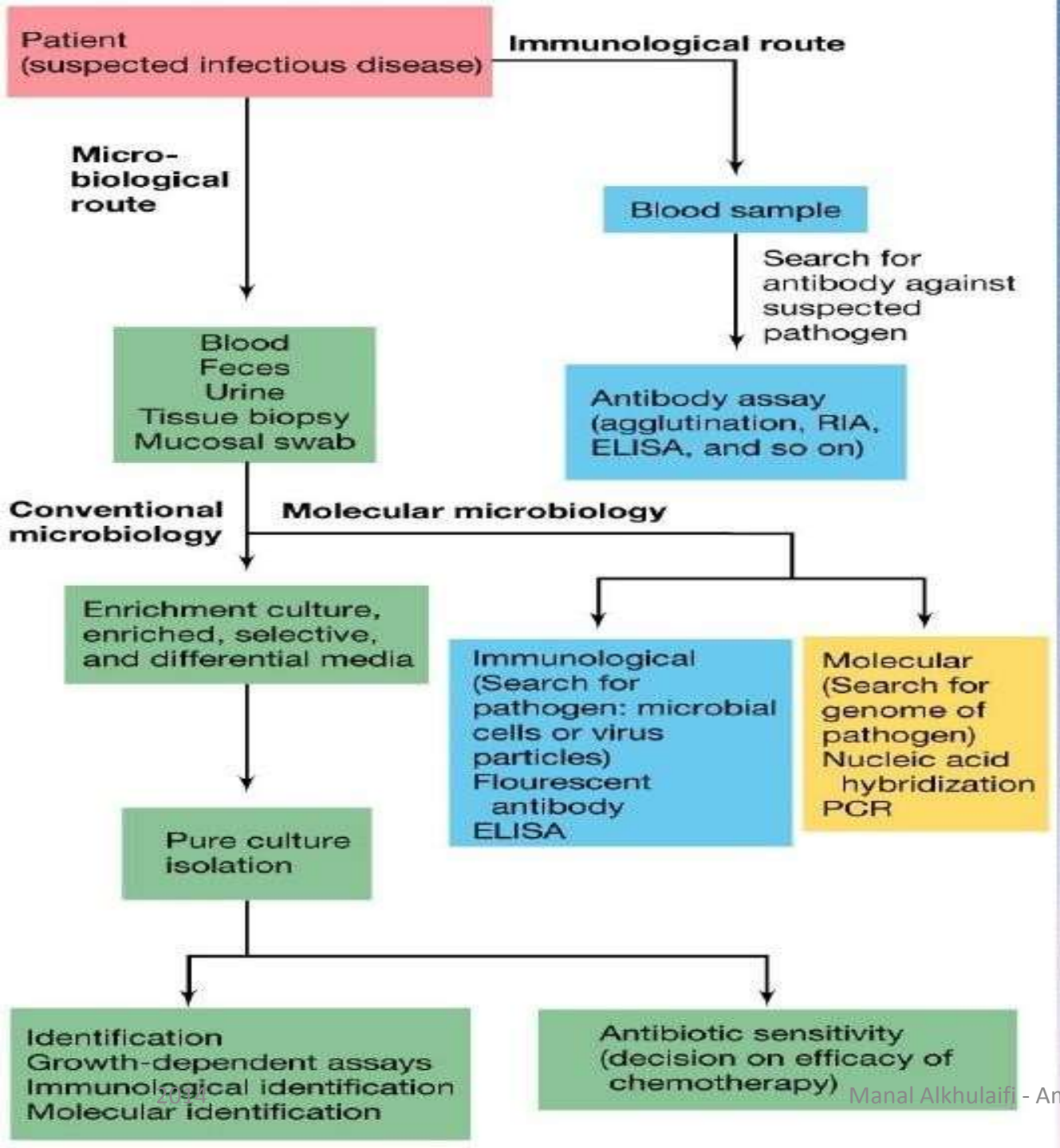


TABLE 13.9 Common Signs and Symptoms of Infectious Diseases

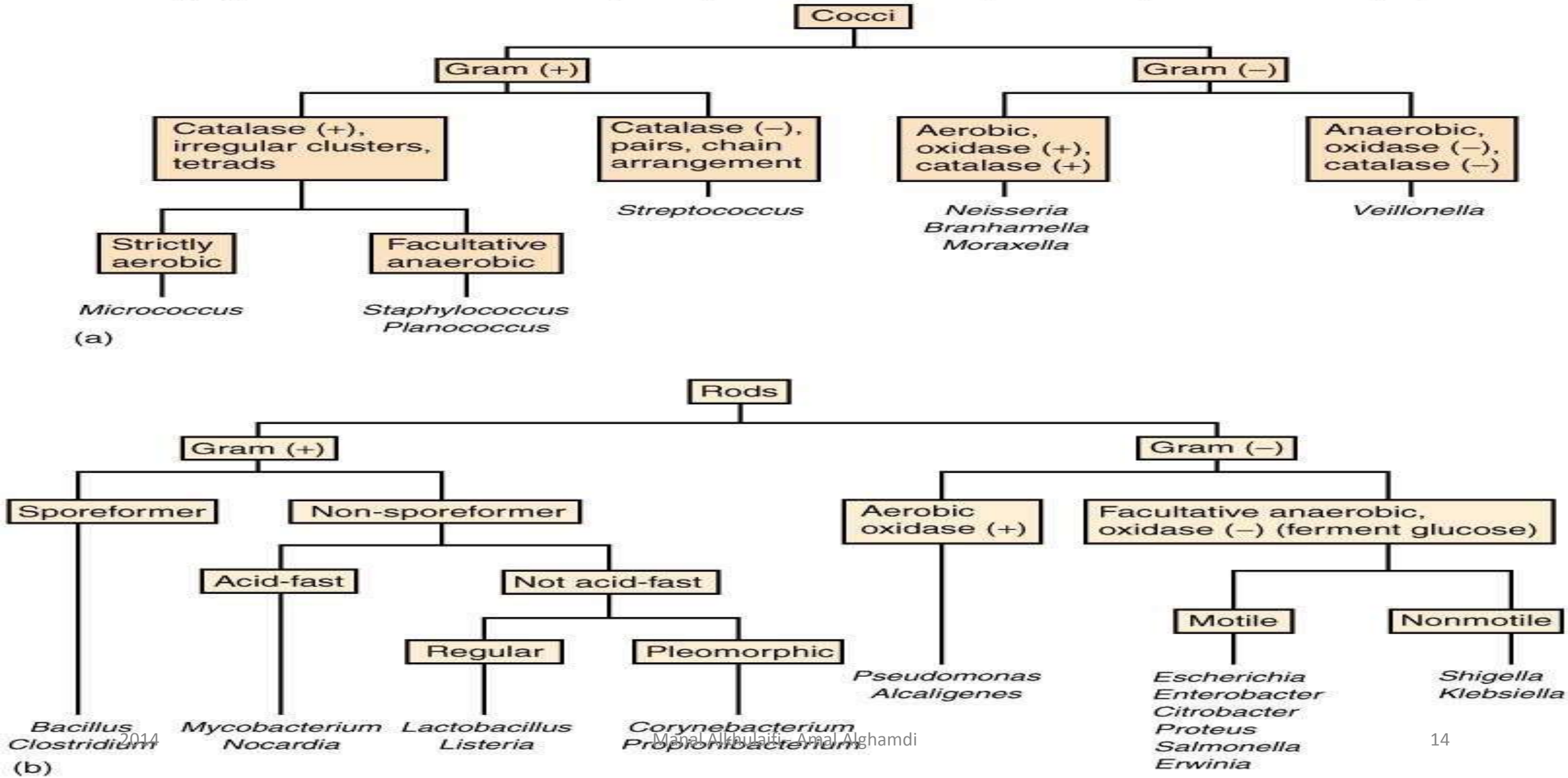
Signs	Symptoms
Fever	Chills
Septicemia	Pain, irritation
Microbes in tissue fluids	Nausea
Chest sounds	Malaise, fatigue
Skin eruptions	Chest tightness
Leukocytosis	Itching
Leukopenia	Headache
Swollen lymph nodes	Weakness
Abscesses	Abdominal cramps
Tachycardia (increased heart rate)	Anorexia (lack of appetite)
Antibodies in serum	Sore throat

- **Signs and Symptoms**
- **Sign**–objective evidence of disease as noted by an observer
- –fever, septicemia, chest sounds, rash, leukocytosis antibodies
- •**Symptom**–subjective evidence of disease as sensed by the patient
- –chills, pain, ache, nausea, itching, headache. Fatigue
- **Infections That Go Unnoticed**
- **Asymptomatic(subclinical) infections –**although infected, the host doesn't show any signs of disease- Inapparent infection, so person doesn't seek medical attention.



Microbe Identification Scheme

Bacterial identification flow chart



Is your investigation worthwhile?

Do you Know what information you want?

No

Yes

Is the information already available?

Yes

No

Does it affect patient management

No

Yes

Can the lab provide this information

No

Yes

Stop !

Think Again

Contact the lab for info on Best Test

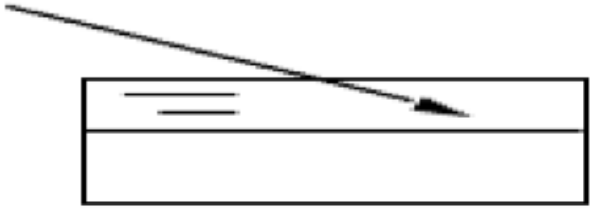
Type of Sample

Timing of Sample

Transport of Sample

Interpretation of Results

Give the Lab. All the relevant clinical info. e.g. Antibiotic treatment
Recent travels
Special Risk



Happy Clinician

Happy Microbiologist

Happy Patient

Happy Manager