

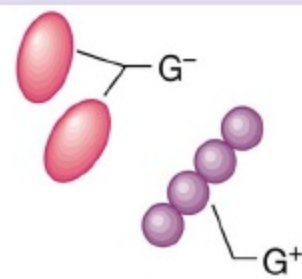
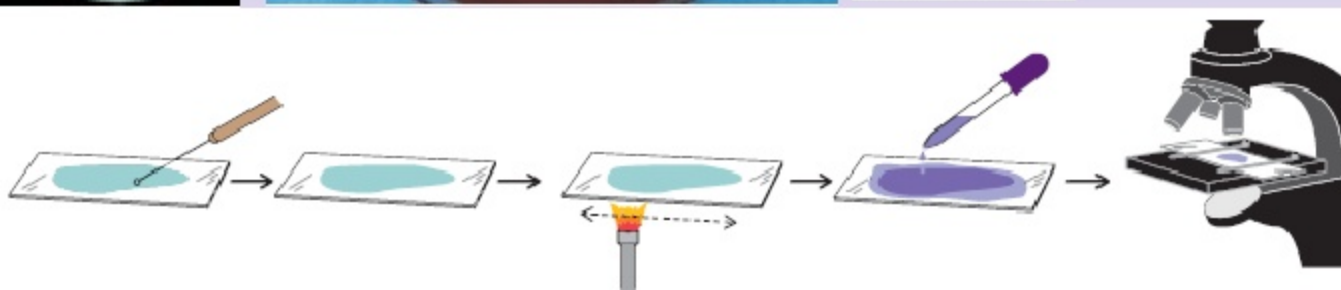
DIAGNOSTIC MICROBIOLOGY

IDENTIFICATION OF MICROBES

Dr. Chhaya Savant

Lecture 3

April 2010



Scope of the present lecture

The importance of studying
microorganisms

An overview of microbe identification
methods

The importance of studying microorganisms

Useful Role

Maintain balance of environment (microbial ecology)

Basis of food chain

Nitrogen fixation

Photosynthesis

Digestion, synthesis of vitamins

Manufacture of food and drink

Bioremediation

Harmful Role

Diseases causing microbes

Identification of Microorganisms

- **How to identify unknown specimens ???????**
- Labs can grow, isolate and identify most routinely encountered bacteria within **48 hrs** of sampling.
- The methods microbiologist use fall into **three categories**:
 - ✓ **Phenotypic**- morphology (micro and macroscopic)
 - ✓ **Immunological**- serological analysis
 - ✓ **Genotypic**- genetic techniques

Phenotypic Methods

- 'Old fashioned' methods via biochemical, serological and morphological are still used to identify many microorganisms.
- **Phenotypic Methods**
- Microscopic Morphology include a combination of cell shape, size, Gram stain, acid fast reaction, special structures e.g. Endospores, granule and capsule can be used to give an **initial presumptive identification**.

Phenotypic Methods

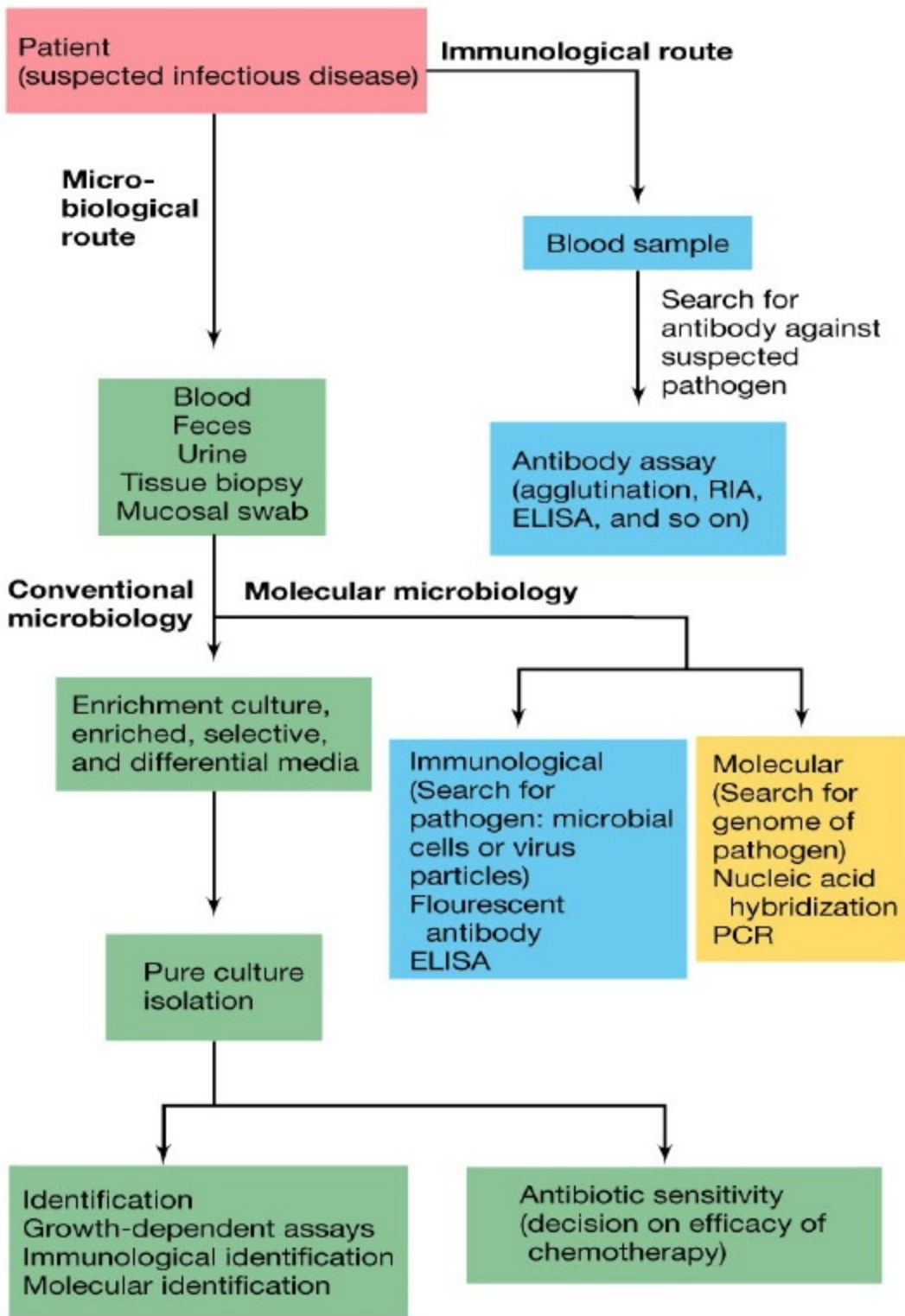
- **Macroscopic morphology** are traits that can be accessed with the naked eye e.g. appearance of colony including texture, shape, pigment, speed of growth and growth pattern in broth.
- **Physiology/Biochemical** characteristic are traditional mainstay of bacterial identification.
- These include enzymes (Catalase, Oxidase, Decarboxylase), fermentation of sugars, capacity to digest or metabolize complex polymers and sensitivity to drugs can be used in identification.

Immunological Methods

- Immunological methods involve the interaction of a microbial **antigen with an antibody** (produced by the host immune system).
- Testing for microbial antigen or the production of antibodies is **often easier** than test for the microbe itself.
- **Lab kits** based on this technique is available for the identification of many microorganisms.

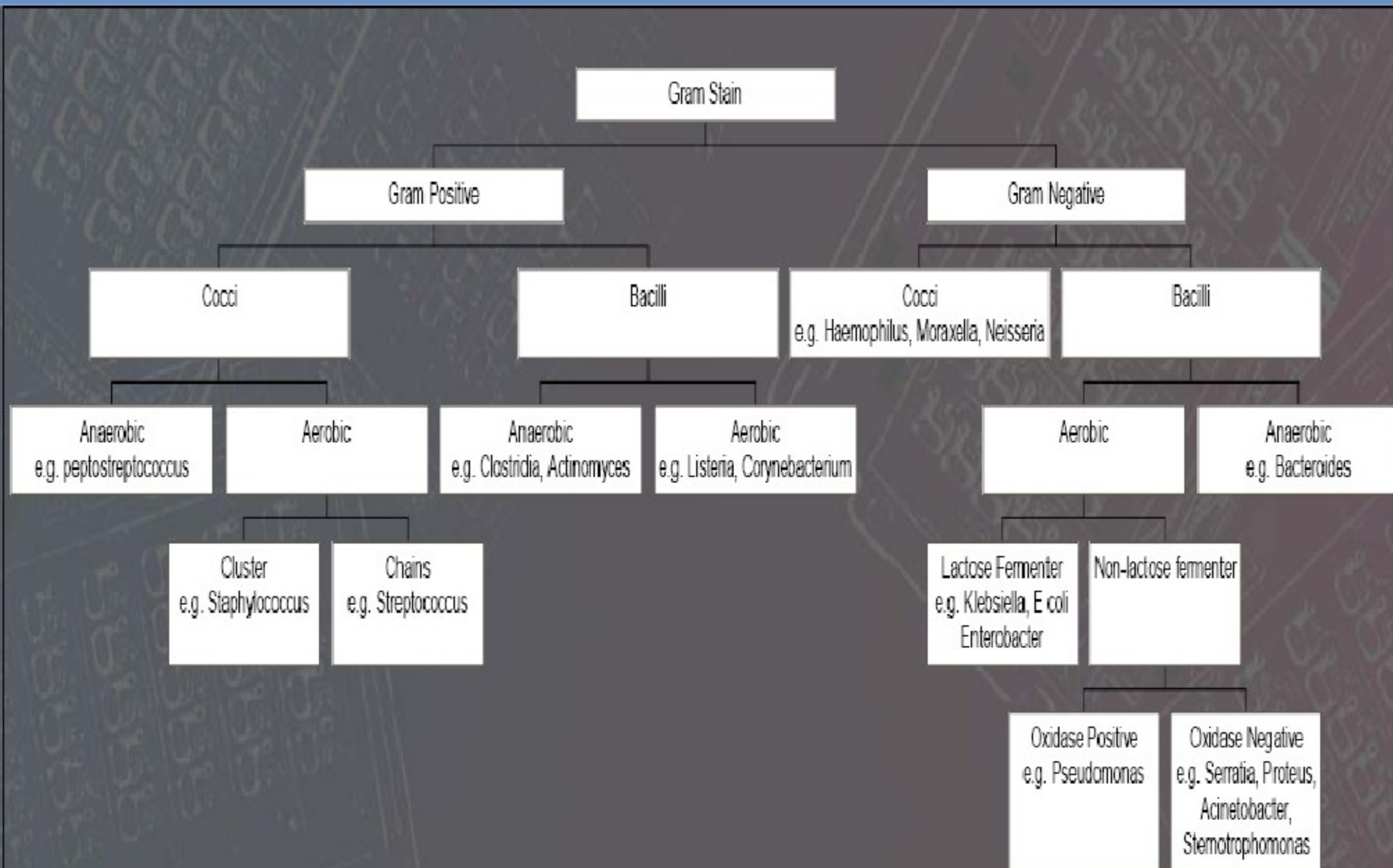
Genotypic Methods

- Genotypic methods involve examining the genetic material of the organisms and has **revolutionized bacterial identification** and classification.
- Genotypic methods include PCR, (RT-PCR, RAPD-PCR), use of nucleic acid probes, RFLP and plasmid fingerprinting.
- Increasingly genotypic techniques are becoming the **sole means of identifying** many microorganisms because of its **speed and accuracy**.



Microbe Identification Scheme

Bacterial Classification



Process

- ◆ Specimen collection
- ◆ Specimen receipt
- ◆ Specimen processing
- ◆ Testing
- ◆ Interpretation
- ◆ Reporting

Microbe Identification

- The **successful identification** of microbe depends on:
 - Using the proper **aseptic techniques**.
 - **Correctly** obtaining the specimen.
 - Correctly **handling the specimen**
 - **Quickly transporting** the specimen to the lab.
 - Once the specimen reaches the lab it is **cultured and identified**
 - Use care and tact to **avoid patient harm**

The specimen is the beginning. All diagnostic information from the laboratory depends upon the knowledge by which specimens are chosen and the care with which they are collected and transported.

—Cynthia A. Needham¹²

Standard Precautions

- Fundamental to safe handling of specimens
- New concept in 1995 - combined 'universal' and 'body substance isolation' precautions
- Applies to all patients irrespective of known or presumed disease status
- Applies to blood, body fluid and body tissue

(Garner 1996)

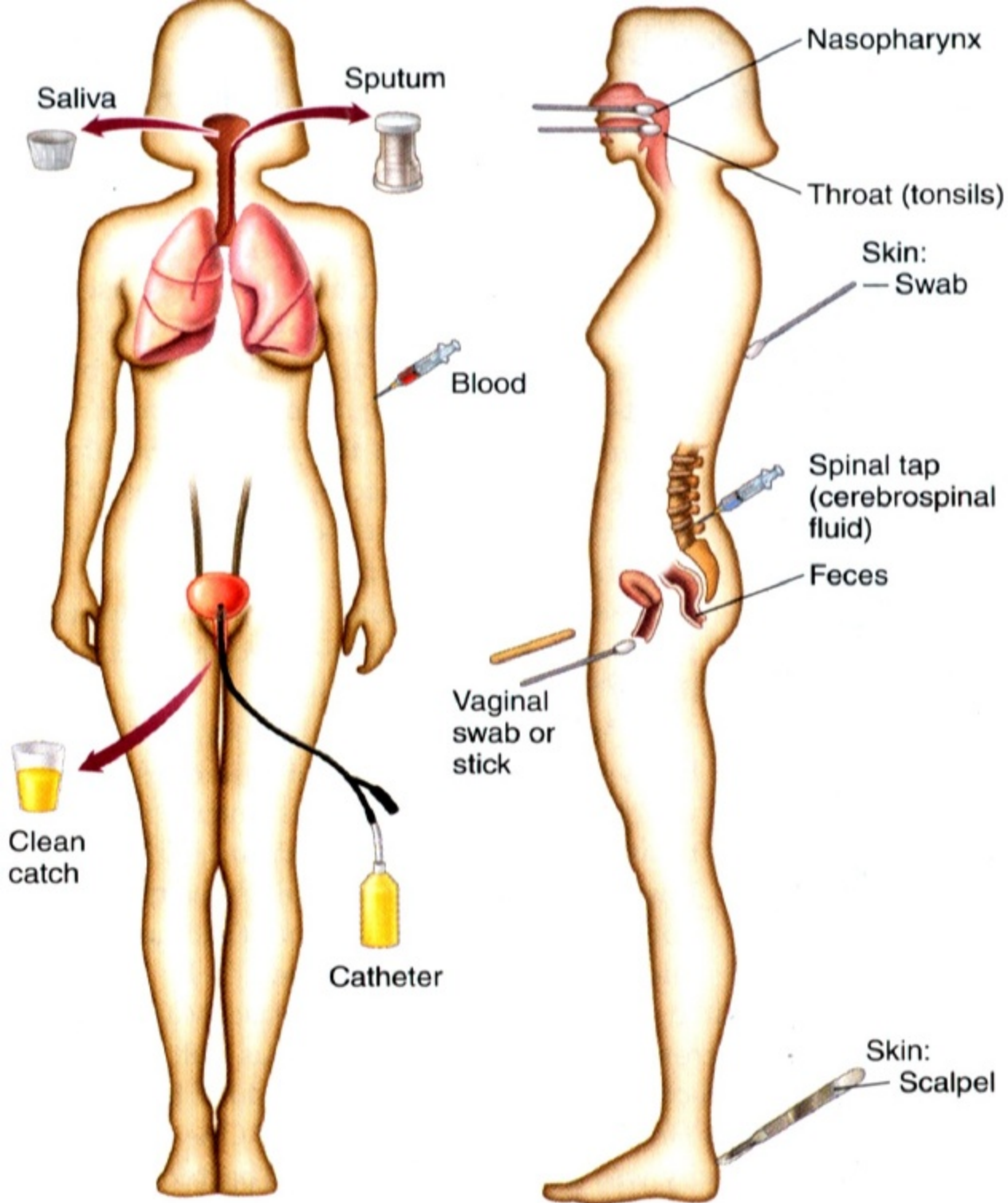
Standard Precautions (cont.)

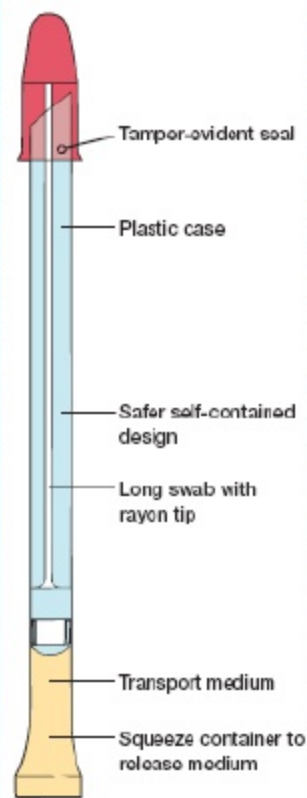
- Hand washing or hand hygiene
 - Antibacterial soap for invasive procedures
 - Waterless alcohol gels or rubs
- Personal Protective Equipment
 - Disposable gloves
 - Disposable aprons / impervious gowns
 - Facial protection – masks, eye goggles
- Safe handling of sharps



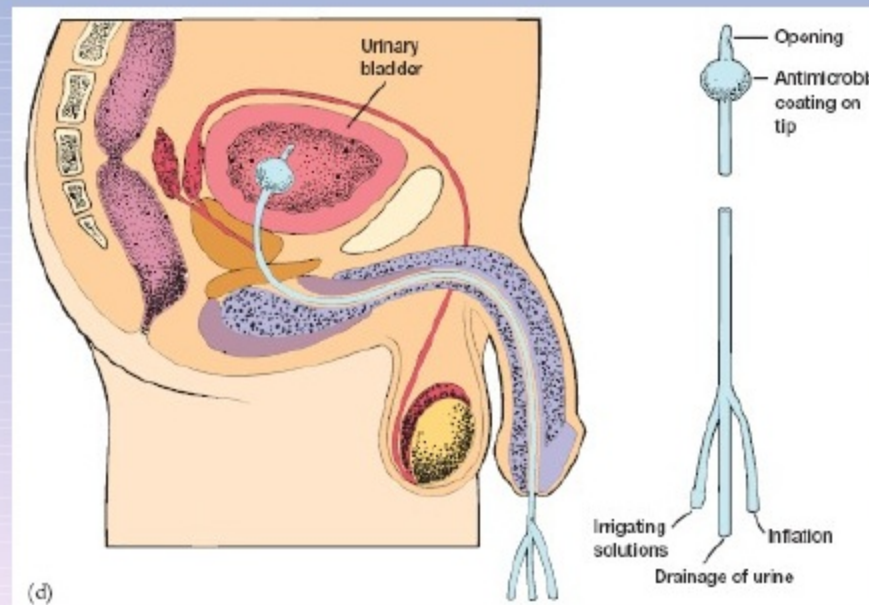
(The National Committee for Clinical Laboratory Standards 2004)

Specimen Collection





- A drawing of a sterile swab with a specific transport medium.
- (b) Sterile Vacutainer tubes for the collection of blood.
- (c) Nasotracheal intubation.
- (d) A drawing of a Foley catheter.
- (e) This specially designed sputum cup allows the patient to expectorate a clinical specimen directly into the cup.



Aerobic/Anaerobic Blood Culture Bottles



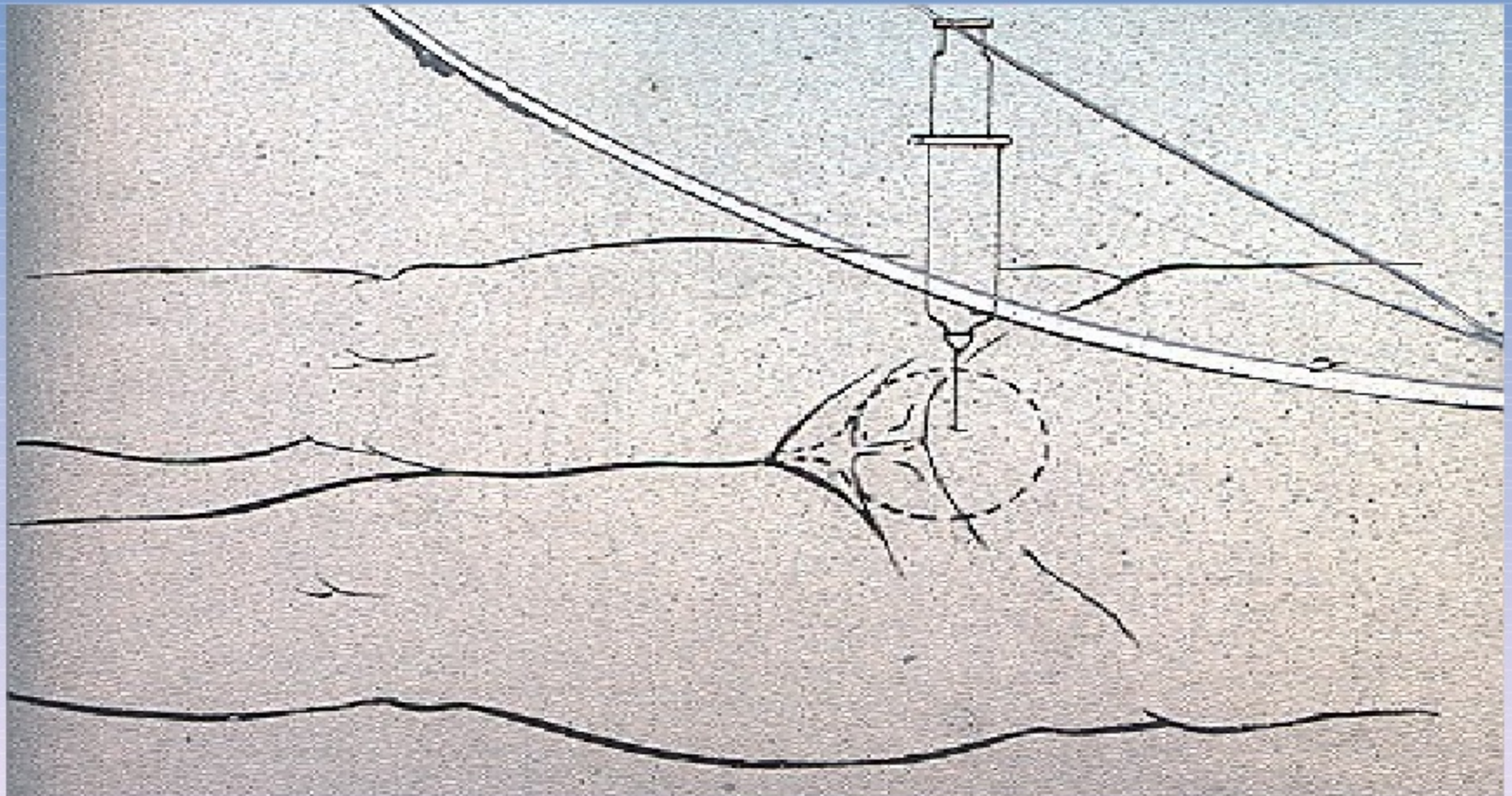
AFB Blood Culture Bottle



Wire Swab



Suprapubic Aspiration



Transportation

- Specimen transported promptly
 - Within 2 hours
- Strict storage conditions
 - for delayed laboratory processing
- Sterile containers
 - Faecal specimens an exception
- Use of transport media
- Provide written guidelines

Microbe Identification

- Identification measures include:
 - ♣ Microscopy (staining)
 - ♣ growth on enrichment, selective, differential or characteristic media
 - ♣ specimen biochemical test (rapid test methods)
 - ♣ immunological techniques
 - ♣ molecular (genotypic) methods.
- After the microbe is identified for clinical samples it is used in **susceptibility tests** to find which method of control is most effective.