ADVANCED LABORATORY TECHNIQUES

SPECIMEN COLLECTION & BACTERIAL DIAGNOSIS (VITEK & BACT)

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third Stage . — المرحلـــــــــة الثالثة

محاضرة رقم 1

Lecture No. 1

الجانب النظري

Theoretical

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(INTRODUCTION)

- Advanced laboratory techniques represent the evolution of diagnostic tools and methods that have transformed clinical laboratories into **highly accurate, automated, and efficient systems**. These techniques are not only extensions of basic laboratory skills but also incorporate innovations in **molecular biology**, **immunology**, and **automation technologies.**
- **Historical Background**
- **Early Phase (19th–20th century):** Diagnostics relied on microscopy, basic culture media, and biochemical tests.
- **Mid 20th century:** The introduction of spectrophotometry, ELISA, and radioimmunoassays allowed more precise quantification.
- **Late 20th century:** Molecular techniques such as PCR (1980s) and flow cytometry expanded diagnostic capacity.21st century: Full automation, digital systems, and integration of artificial intelligence have reshaped laboratory medicine.

(INTRODUCTION)

- **Main Advanced Techniques** :

➢ **Molecular Biology Techniques**: PCR, RT-PCR, Real-time PCR, DNA sequencing.

➢ **Immunological Techniques**: ELISA, Western blotting, Chemiluminescent assays.

➢ **Cellular Analysis:** Flow cytometry, Fluorescent microscopy.

➢ **Automated Systems**: Blood culture systems, automated identification, and antimicrobial susceptibility testing.

- **Applications in Modern Diagnostics:**

Advanced techniques are widely applied in:

➢ Microbial diagnostics (identification of bacteria, viruses, fungi).

➢ Cancer diagnostics (tumor markers, genetic mutations).

➢ Immunological disorders (autoantibodies, cytokine profiling).

➢ Personalized medicine (genomic sequencing for targeted therapy).

Specimen Collection

Specimen Collection:

specimen Collection Is **The Process Of Obtaining Biological Samples From A Patient (Such As Blood, Urine, Stool, Swabs, Or Body Fluids) For Laboratory Examination.** The Accuracy Of Laboratory Diagnosis Highly Depends On **The Quality Of The Specimen** And The **Method Of Collection.**

General Principles:

- ✓ **The specimen must be representative of the infected site.**
- ✓ **Aseptic technique is essential to avoid contamination.**
- ✓ **Use the appropriate container depending on the type of specimen.**
- ✓ **Always label specimens with full patient information (name, age, file number, type of specimen, and time of collection).**
- ✓ **Transport specimens to the laboratory as soon as possible or store under the required conditions (refrigeration, preservatives, etc.).**

Specimen Collection

Transportation Of Specimens

- **csf:** Must Be Examined Immediately; Do Not Refrigerate.
- **Blood**: Usually Kept At Room Temperature Unless Otherwise Specified.
- **Urine And Stool:** May Be Refrigerated If There Is A Delay In Processing.
- **Swabs**: Should Be Placed In Appropriate Transport Media.

BACTERIAL DIAGNOSIS:

Bacterial Diagnosis Is The Cornerstone Of Clinical Microbiology, Aiming To **Identify The Presence, Type, And Characteristics Of Bacteria** Responsible For Human Diseases. Accurate And Rapid Diagnosis Is Critical Because It Guides Clinicians In Selecting The Most Effective **Antimicrobial Therapy**, Prevents Complications, And Supports Infection Control.

- **Importance**:
- Early detection of bacterial pathogens reduces morbidity and mortality.
- Helps in monitoring antimicrobial resistance patterns.
- Provides essential information for epidemiological surveillance and outbreak managemen

VITEC SYSTEM

“Vital Technology”, Representing An Essential Technology For Microbiological Diagnostics. It Is One Of The Most Widely Used **Automated Systems** In Clinical Microbiology.

WHAT DOES THE VITEK SYSTEM DO?

- **BACTERIAL IDENTIFICATION (ID):**
  - Uses small plastic cards containing more than **60 miniaturized biochemical tests**.
  - After inoculating the card with the bacterial suspension, the system reads the biochemical reactions (colorimetric/fluorometric).
  - Results are compared with a large database to identify bacteria at the **species level**.

- **ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST):**
  - Determines bacterial susceptibility or resistance to antibiotics.
  - Provides **Minimum Inhibitory Concentration (MIC)** values.
  - Guides clinicians in selecting the most effective therapy.

Applications:

- ✓ Clinical microbiology laboratories: Used for diagnosing bloodstream infections, urinary tract infections, respiratory infections, and wound infections.
- ✓ Hospital infection control: Surveillance of antimicrobial resistance (AMR) patterns
- ✓ Food and water microbiology: Occasionally applied for detecting bacterial contamination in food and beverages.
- ✓ Pharmaceutical industry: Quality control to ensure products are free from microbial contamination.

Advantages

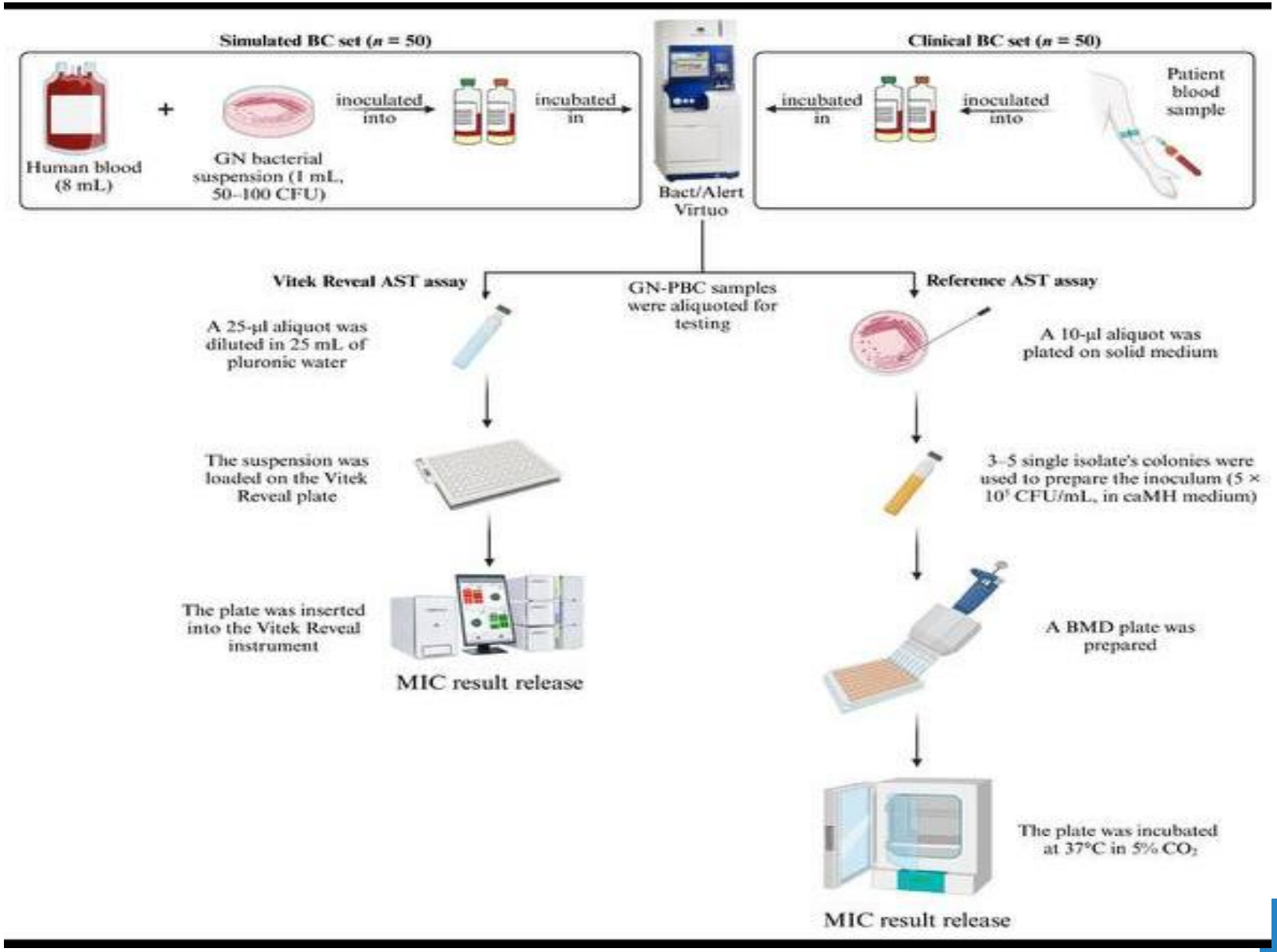
- **Rapid:** Results Within Hours Compared To Days With Traditional Methods.
- **Accurate:** Supported By A Large And Updated Database.
- **Efficient:** Reduces Manual Workload And Increases Laboratory Productivity.

BACT AUTOMATED SYSTEM

A "BACT automated system" refers to the BacT/ALERT® automated microbial detection systems, produced by bioMérieux. These systems are designed for continuous, automated monitoring of cultures in bottles to detect the presence of bacteria and fungi in blood, body fluids, and industrial products. The technology relies on measuring carbon dioxide (CO2) production through a colorimetric sensor, with faster and more efficient results compared to traditional methods

Purpose: Detects The Presence Of Microorganisms, Including Bacteria And Fungi, In Various Clinical Samples, Such As Blood.

Methodology: Employs Culture Bottles With Built-in Sensors That Detect The Production Of Gases Or Changes In Fluorescence Caused By Growing Microorganisms.


Simulated BC set (n = 50)
Human blood (8 mL)
+
GN bacterial suspension (1 mL, 50–100 CFU)
inoculated into
incubated in
Bact/Alert Virtuo
Clinical BC set (n = 50)
incubated in
inoculated into
Patient blood sample
GN-PBC samples were aliquoted for testing
Vitek Reveal AST assay
A 25-µl aliquot was diluted in 25 mL of pluronic water
The suspension was loaded on the Vitek Reveal plate
The plate was inserted into the Vitek Reveal instrument
MIC result release
Reference AST assay
A 10-µl aliquot was plated on solid medium
3–5 single isolate's colonies were used to prepare the inoculum (5 × 10⁵ CFU/mL, in caMH medium)
A BMD plate was prepared
The plate was incubated at 37°C in 5% CO₂
MIC result release

BACT AUTOMATED SYSTEM

How the BacTSystem Works:

Sample and Bottle Preparation:

A sample (e.g., blood) is added to a specialized BacT bottle containing a liquid sensor.

Colorimetric Detection:

The bottle contains a pH-sensitive sensor that changes color in response to CO2 levels.

Continuous Monitoring:

The system incubates and continuously monitors the bottles, taking readings of the sensor every 10 minutes.

Alerting:

If a significant color change is detected, it signals the presence of microorganisms, and the system provides an alert to the user.

Advantages:

Automation:

The System Fully Automates The Process Of Incubating, Shaking, And Monitoring Culture Media.

Speed:

It Provides Rapid And Continuous Monitoring, Allowing For The Faster Identification Of Microbial Contamination.

Versatility:

The System Is Used In Various Settings, Including Clinical Microbiology Labs For Detecting Bloodstream Infections And In Industrial Labs For Product Sterility Testing.

Efficiency:

Automation Reduces Hands-on Time For Laboratory Personnel, Improving Efficiency And Allowing For More Rapid Reporting Of Results.

Reliability:

The Bact System Is A Proven And Reliable Tool For Detecting A Wide Variety Of Bacteria And Fungi.

Q/the differences between two techniques :

The Primary Difference Is That The Bact/ALERT System Is A Blood Culture Instrument That Uses Continuous Monitoring To Detect Microorganisms, While The VITEK System (Often Also Automated) Is Used For The Subsequent Identification Of Bacteria And Antibiotic Susceptibility Testing (AST) After They Have Been Isolated From A Culture. The Bact/ALERT System Identifies The Growth Of Microbes, And The VITEK 2 System Then Uses The Isolated Bacteria To Determine Which Species It Is And Which Antibiotics Will Be Effective Against It

QUIZ

H.W:

What Are The Main Differences Between Traditional Bacterial Diagnosis Methods (Like Culture And Microscopy) And Advanced Techniques?

Any questions?