

Session 2: Molecular Microbiology

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Learning Outcomes

1. Identify appropriate molecular methods used in pathogen genomics
2. Apply molecular approaches and techniques used in microbiology, outbreak detection, surveillance and basic research
3. Understand the key components and infrastructure required to support molecular microbiology workflows

Course Content

- I. Importance of molecular microbiology in pathogen genomics
- II. Molecular biology tools and applications
- III. Overview of molecular microbiology infrastructure, equipment, and personnel requirement

Importance of molecular microbiology in pathogen genomics

- The function of a clinical microbiology laboratory is to identify pathogens using direct observation of microbes and culture-based methods:
Expensive, Insufficient & Tedious
- Molecular techniques are useful in high-quality microbiology labs

- Detection of pathogens
- Guide clinical decisions
- Monitor the effectiveness of therapeutics
- Mutation screening
- Outbreak detection
- Laboratory Surveillance
- Gene expression studies
- Sequencing

Molecular biology tools and applications

Non-amplified DNA probe-based methods

- Clinical diagnostic
- Monitoring of therapeutics
- *In situ* hybridization

Nucleic acid amplification

- Pathogen detection & mutation screening
- PCR, M-PCR, RT-PCR
- LAMP, NASBA

Molecular typing methods

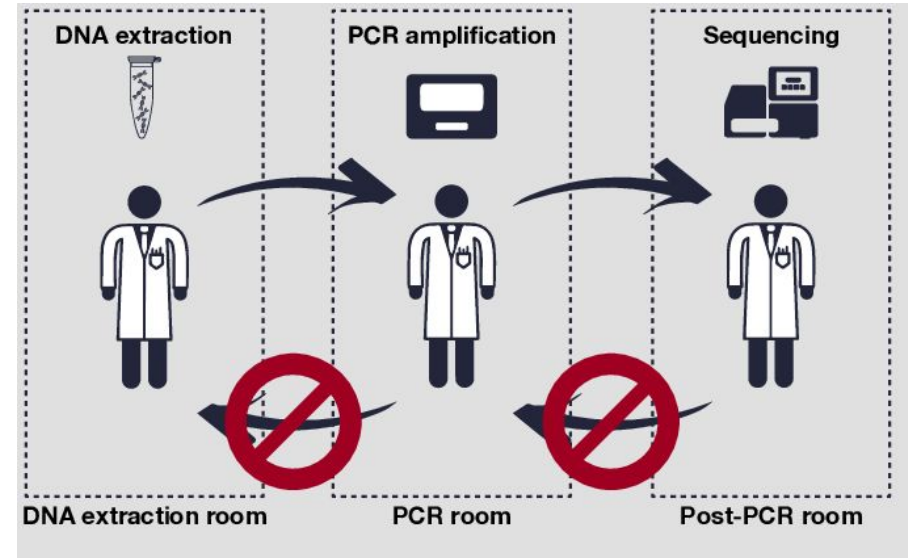
- Outbreak detection
- PFGE, Ribotyping
- Nucleic acid based-typing schemes

New molecular methods

- Clinical diagnostic
- Monitoring of therapeutics
- Real-time droplet PCR, Mass spectrometry

Overview of molecular microbiology laboratories

- When establishing molecular capacity, it is important to consider:
 - Applications
 - Laboratory design \Rightarrow Infrastructure
 - Laboratory practices \Rightarrow personnel
 - Equipment, Chemical and enzymatic controls

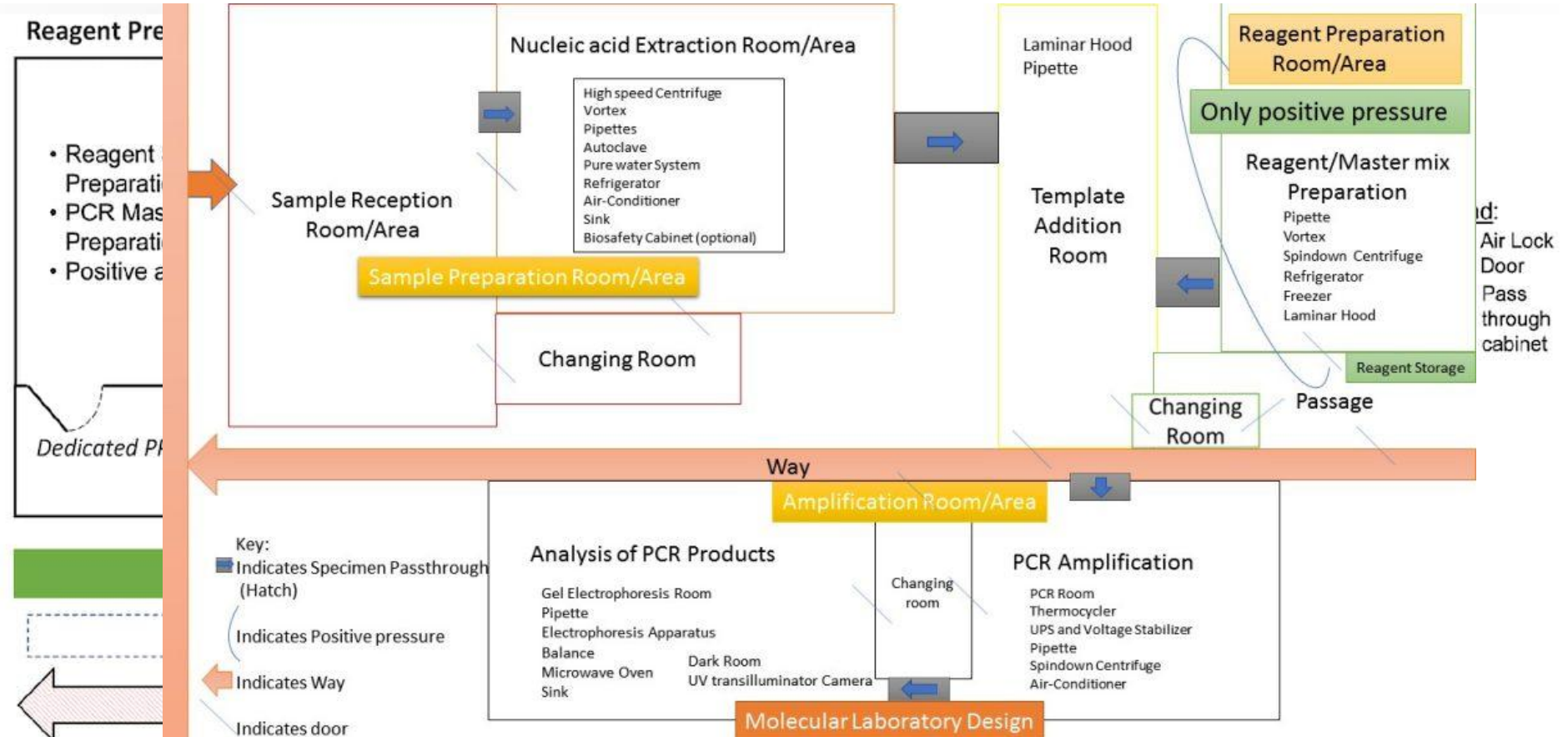


Overview of molecular microbiology laboratories: Infrastructure

- **Setting Up a Molecular Laboratory**
 - **A clean and well-controlled environment**
 - Mechanical barriers to prevent contamination
 - Spatial separation of pre- and post-amplification
- **At least 3 work areas**
 - Area 1 – Reagent preparation
 - Area 2 – Specimen/control preparation, PCR set-up
 - Area 3 – Amplification/product detection, plasmid preparation
- **Unidirectional workflow - pressure**
 - Avoid or limit the reverse direction
 - Both personnel, including cleaning personnel, and specimens
 - Amplification from product-free to product-rich
 - Remove PPE before leaving one area
- **Separated sets of equipment and supplies**

Each area has separated sets of equipment and supplies

Establishing Capacity for Pathogen Genomics
Addis Ababa, Ethiopia, May 2023



Size of each area should consider space for equipment and bench space needed

<https://universe84a.com/molecular-laboratory-set/>

Overview of molecular microbiology laboratories: Personnel

- Laboratory personnel should be adequately trained on :
 - Basic training including liquid handling, sample processing, etc.
 - All safety measures: Biosafety management programme with regular training schemes
⇒ensuring the use of standard practices and procedures.
 - An effective laboratory program should be introduced by integrating safe laboratory practices into the basic training of the personnel
- Evaluation and accreditation ⇒ recognition that the laboratory can perform quality experiments
- Management of the physical laboratory infrastructure and equipment

Overview of molecular microbiology laboratories: Equipment

Do's — Favour equipment

- **Cost-effective, Simple, user-friendly**
- Robust in LMICs
- With on-site training by the manufacturer
- Documented procedures for the maintenance, calibration & validation
- Local manufacturer representative

Dont's — Avoid equipment requiring

- Service contracts
- Calibrations from manufacturers' technicians
- Full automation
- Important energy supply
- Manufacturer representatives from overseas

Overview of molecular microbiology laboratories: Quality control

- Strict quality control & quality assurance programs
 - Evaluation and accreditation ⇒ recognition that the laboratory can perform quality experiments
 - Document all procedures and assays
 - Ensure use of control in all experiments
- **Blank reaction**
 - Controls for contamination
 - Contains all reagents except DNA template
 - **Negative control reaction**
 - Controls for the specificity of the amplification reaction
 - Contains all reagents and a DNA template lacking the target sequence
 - **Positive control reaction**
 - Controls for sensitivity
 - Contains all reagents and a known target-containing DNA template
 - **Internal control reaction**
 - Controls for the presence of inhibitors and PCR efficiency

Take-home messages

- A high-quality microbiology laboratory may include both conventional and molecular microbiology techniques for exceptional performance.
- Numerous molecular methods exist with various applications
- Setting up a molecular microbiology lab requires:
 - Physical infrastructure with at least 3 dedicated working areas
 - Trained personnel
 - Provision of equipment and maintenance
 - Supplementation of consumables and reagents

THANK YOU

Questions?

Comments?

Suggestions?

Real world exercise

Africa CDC have given you a grant of USD 500,000 to set up a molecular microbiology laboratory training facility at your institution. You have been giving a building of 200 m² (10m by 20m).

1. Can you outline the key priorities and design of your facilities?
2. Think about the building itself, equipment, consumables/materials/reagents, personnel, security, etc. you will need to have in your facility.

Real world exercise - guidance

- You have been randomly split into six groups
- Use the tickets on the shopping list to build your lab.
- Choose all that apply, and take into consideration the price and the funds provided
- Use the provided blank sheets, pencils, rulers, etc., to draw your molecular microbiology lab
- Use PowerPoint or flipchart to :
 - Summarize the infrastructure, activities, assays and team in your lab – one slide
 - Justify the items you bought – one slide/sheet