Day 2: Specimen and sequencing

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COG-TRAIN
COVID-19 GENOMICS GLOBAL TRAINING

Course roadmap

Sun 7 May
Introduction Day

Mon 8 May
Day 1
Capacity Building

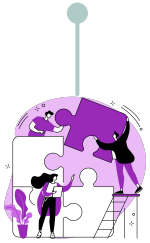
Tue 9 May
Day 2
Specimen and Sequencing

Wed 10 May
Day 3
Data Tools and Pipelines

Thu 11 May
Day 4
Frameworks, Guidelines, and Decision-making

Fri 12 May
Day 5
Projects Review and Action Planning

Next steps and Beyond



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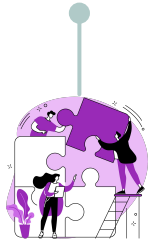

Specimen collection and processing
Molecular microbiology
Sequencing infrastructure

Summary of Day 2

8:30 Session 1 - Specimen and data collection and processing

10:30 Coffee break

11:00 Session 2 - Molecular microbiology

12:30 Session 3 - Setting up genomic sequencing infrastructure

13:00 Lunch

14:00 Resume to Session 3 - Setting up genomic sequencing infrastructure

15:00 Session 4 - Translating to own contexts I- sample to sequencing

16:00 Coffee break

16:30 Session 5 - Design genomics training II - Specimen to sequencing

Session 1: Specimen and data collection and processing

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Part 1 - Sample collection

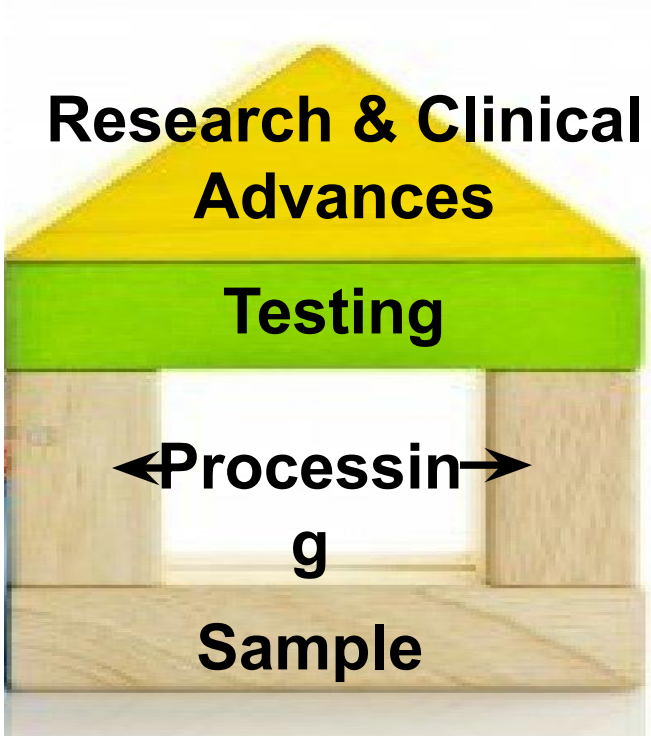
Objectives

By the end of this session, you should be able to describe the process of:

- Differentiate the types of specimen for each purpose
- Explain the importance of the proper collection of human biological samples and its effect on the quality and integrity of testing result.

Importance of specimen collection

- Provides foundation of all downstream activities
- Primary resource of biorepositories
- Source of research discoveries
- Source of patient advancement
- Potential to inhibit analysis



Importance of proper specimen collection and handling

It is a foundational principle for any laboratory test procedure that the value of the test is compromised or even negated by using specimens that have not been properly collected, labelled, handled or stored prior to and during the testing process.

The accuracy of results leads to accurate diagnostic and therapeutic decisions. **To ensure maximum accuracy of results** proper patient preparation, specimen collection and handling are necessary. This requires the timely provision of properly collected specimens to the laboratory.

Erroneous results as a result of specimen mis-management can affect patient care and outcomes, as well as hospital infection control,costs and laboratory efficiency

Ethics

- Recruitment
- Incentives
- Informed Consent
- Confidentiality
- Safety

Decisions: Sample Types

- Project goals
- Future research
- Innovation
- Feasibility
- Costs
- Processing
- Storage
- Stability

Sample Collection: General Processes

- Requisition form
- Register
- Collection Supplies
- Verify Identity
- Label tube/container
- Prep Client
- Collect Sample
- Temporary Storage

Procurement of Supplies

Vendor/Supply Company

- Identify a vendor/supply company - local/international
- Register and have an account with the vendor/supply company to benefit from discounts.

Challenges

- Currency exchange rate
- Supply procurement process and cost – e.g Covid-19

Questions

Part 2 - Sample processing

Objectives

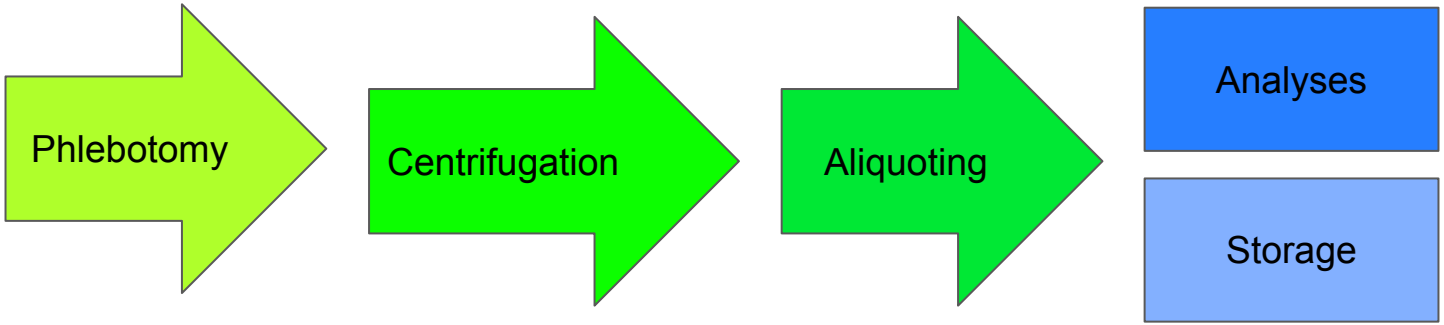
At the end of this session, participants will be able to:

- Identify the different types of equipment used for specimen processing
- Understand the importance of appropriate sample handling and storage

Introduction

- Properly collected and processed specimens represent a very important step for the laboratory and laboratory testing.
- The accuracy of all laboratory test results depends on the quality of the specimen submitted.
- Laboratory results are only as good as the specimen
- Specimen quality is dependent on collection, transportation, processing and storage

General blood separation processing



Equipment & Supplies

	Plasma/Serum Preparation
1	PPE's: Lab coats, Gloves, goggles
2	Appropriate vacutainer tubes
3	Serological pipettes of appropriate volumes (sterile)
4	Pipette Aid, micropipette or sterile transfer pipette of appropriate volume
5	2ml screw cap Cryovials (See study laboratory or specific procedure for appropriate brand, size and type)
6	Benchtop centrifuge with swing out rotor and appropriate carriers and RPM (14,000 rpm)
7	Biohazard Safety Cabinet
8	Mechanical Pipettes and Tips of appropriate volume

Sample handling

- Always apply aseptic techniques when handling samples: from collection to storage. This will avoid contamination and degradation of the samples.
- Aliquot appropriate volumes according to manufacturer's or your laboratory protocols
- Use appropriate plasticware and materials according to your laboratory quality management system
- Store samples according to relevant protocols
- Keep records of the sample processing chain, e.g.: who processed them, when they were collected and handled, where they are stored, etc.

Summary

- Quality laboratory results begin with proper collection and handling of the specimen submitted for analysis

- Specimen integrity can be ensured when appropriate specimen management and transportation procedures are established and followed


ANY
QUESTIONS?

Part 3 - DNA Extraction and QC

Objectives

At the end of this part you will be able to:

- Describe the principles applied for DNA extraction
- Describe the methods used for DNA quality control

Sources of DNA

DNA is isolated from a variety of sources including:

Plants, Animals, Bacterial Cells, Human DNA from Red blood cells (whole blood)

Basic Principle

Cellular content (containing DNA) and other biomolecues (RNA, Protein) needs to be accessed and DNA extracted

<u>How do you access cellular content?</u>

Physical/Mechanical (Freeze- thaw and Sonication)

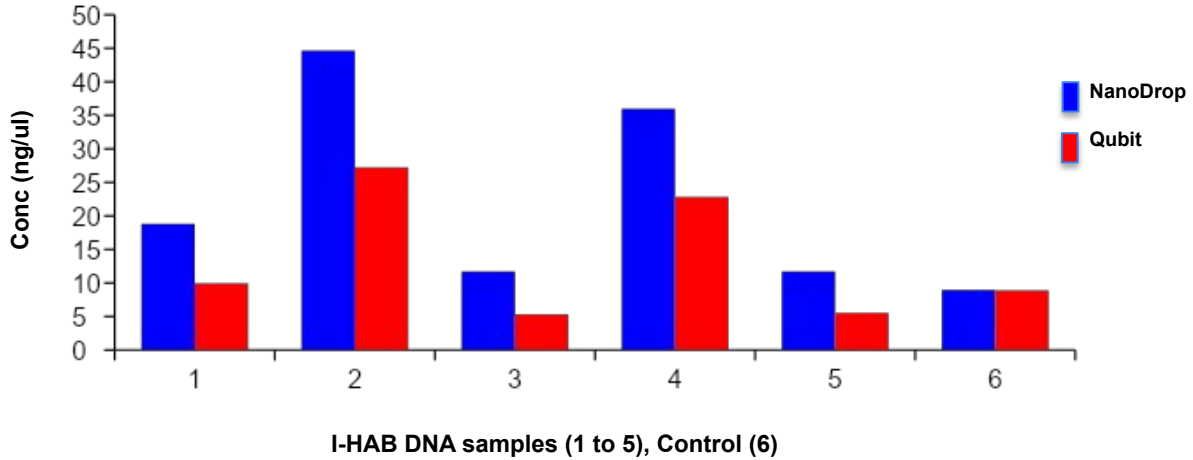
Chemical methods

- Bacterial cell wall – Lyse using lysozyme
- Lipid bi-layer membrane is disrupted using Detergents
- EDTA to remove metal ions that bind components of the outer membrane together

DNA QC assessment tools

- Spectrophotometry (Absorbance at 260 nm (A_{260})
- *Qubit Fluorometer*
- Agarose gel electrophoresis (visualization of cut and uncut DNA) for sample integrity

NanoDrop and Qubit DNA Concentration



DNA Quality Control

Discussion questions:

1. **Why perform quality control (QC) on samples for storage?**

1. **Which of the below equipments you have in your laboratory?**
   a. Spectrophotometry (Absorbance at 260 nm (A_{260})
   b. Qubit Fluorometer
   c. Agarose gel electrophoresis (visualization of cut and uncut DNA) for sample integrity

1. **What are their applications and limitations?**

Part 4 - Sample storage

Objectives

At the end of this session trainees would be able to:

- Describe the workflow for sample archiving
- Implement records and documentation chain
- Understand the importance of temperature monitoring for sample long-term preservation

Introduction

Sample archival:

- Refers to long term storage of samples.
- Supports future sample use for new studies, technologies, theories, etc.
- Enables broader investigations & collaborations

Archiving Samples

- Sample receipt
- Record keeping
- Freezer organization and sample archival
- Temperature Monitoring
- Computer Technology

Sample Receipt

Compare information on sample to paperwork

Report discrepancies

Visually observe sample integrity (if applicable)

View labels & documents for required elements

Apply acceptance/rejection criteria

Compromised sample integrity?
Mislabeled or unlabeled samples?
Incomplete information?

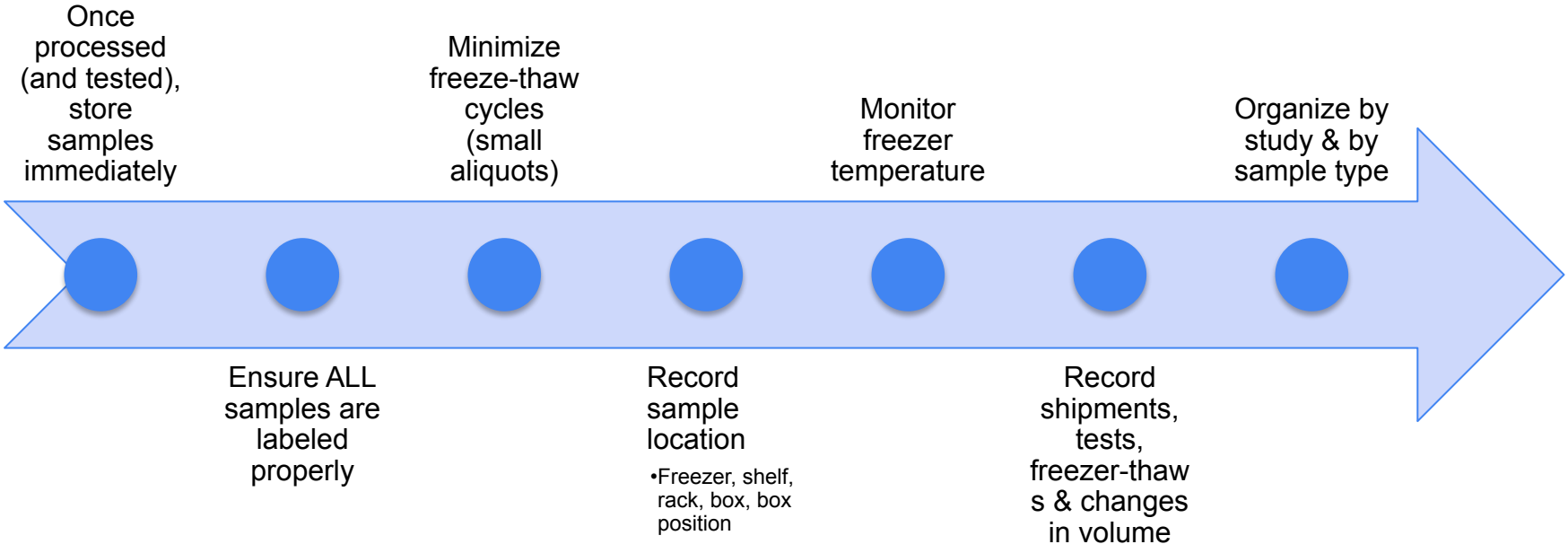
Record Keeping

1. Log data into a Specimen Receipt Logbook/register by date of receipt and in chronological order
2. Fill in as much information as possible
3. Document assigned storage position
4. Note any discrepancies

Storage Considerations

- Optimal Temperature
- Storage receptacle (leak proof, stable at storage temperature, stable over time)
- Storage volume & additive (where apply)
- Secondary container for storage (Ex. Box)
- Rate of cooling (cells)
- Temperature gradient in freezers, LN, etc.
- Back up storage
- Back up power
- Monitoring

Archiving Specimens



Organization of Freezers

Freezers

- Label with numbers, projects , other unique ID

Freezer Shelves (if applicable)

- Establish order of numbering
- Label with numbers or letters

Freezer Racks

- Establish order of numbering
- Label with numbers

Freezer Boxes

- Label with numbers, (study), contents

Documentation

Manual or LIMS-generated box map

Freezer, LN, Incubator, Refrigerator ID
Shelf #
Rack # or letter
Box
Cell

Temperature Charts

Note: Maintenance Charts, Repair Docs, Contracts & Corrective Actions are also relevant to freezers

Temperature monitoring

Temperature should be checked and recorded twice daily

Remote Temperature temp monitoring also essential (Tutela/smartvue)

Temperature Charts should include:

- Ambient, all refrigerators and freezers in lab
- All days in the month
- Acceptable temperature ranges

Summary

Proper organization, records, and temperature monitoring are essential to repository quality.

Sample archiving is of importance to the advancement of research & public health.

Sample archiving saves time & money compared to attaining new samples.

The integrity of the samples must be maintained to ensure usefulness.

Acknowledgement

I-HAB team

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Thank
You!

Group activity

Sample Storage

- Introduction of activity
- Groups will be split into 2
    - Group 1: Case study (Sample receipt)
        - A facility sends a sample to your lab for processing and storage. You notice a discrepancy between the sample manifest and the actual sample. What steps do you take to resolve the problem?
    - Group 2:Case study (Acceptance criteria)
        - A sample that doesn't meet the acceptance criteria was delivered to your lab. What actions do you take to deal with the circumstance?

-Discussion and presentation by groups

Case study 1: Our Approach

- **Discordance**
  - Fill query form
  - Report all findings to supervisor/Manager within 24hrs
  - Manager/Designee to report back findings to shippers as follows; within 1-3 days on shipment nonconformities.

Case study 2 : Our Approach

- Log In all rejected samples into your laboratory register/log and LIMS.
- Notify the Laboratory Supervisor/and or manager within 24 hours
- Document flagged samples on the query form for samples and rejected on the specimen rejection form
- Supervisor/Manager to notify the Requestor/ Shipper within 2-3 days
- Request another specimen
- In cases where the Laboratory Supervisor allows acceptance of an apparently unacceptable specimen, have the Supervisor initial the documentation of acceptance
- The Laboratory Supervisor will determine the need to provide information along with the result report as to the issues surrounding the investigation and the possibility that the specimen may have been compromised before testing