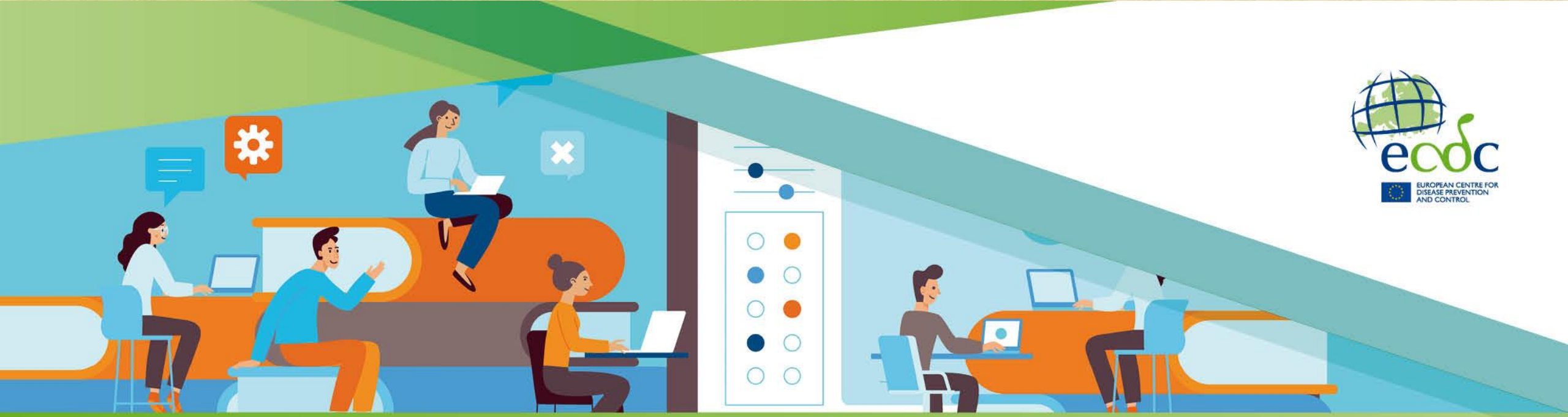


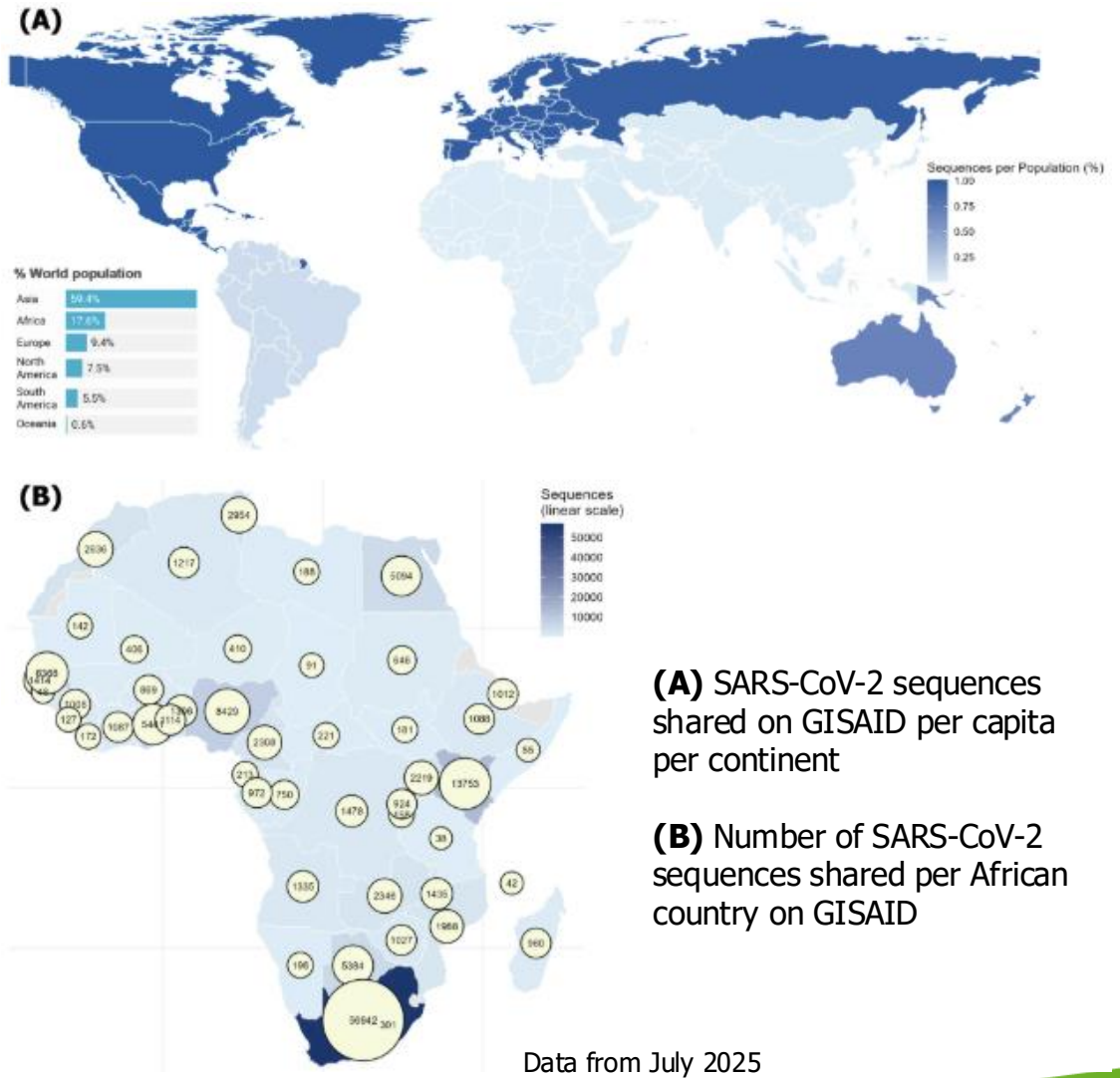
Session 2: Exploring the challenges of performing Mpox WGS in LMIC settings

12/10/2026

Dr. Hayley Cassidy

Why is WGS capacity so important in LMIC?

- Capacity to perform genomic surveillance at scale is mainly present in high-income countries.
- Reflects not only technical gaps but also different priorities, as well as broader structural and socioeconomic challenges inherent to the region.
- If we use SARS-CoV-2 as a case study….



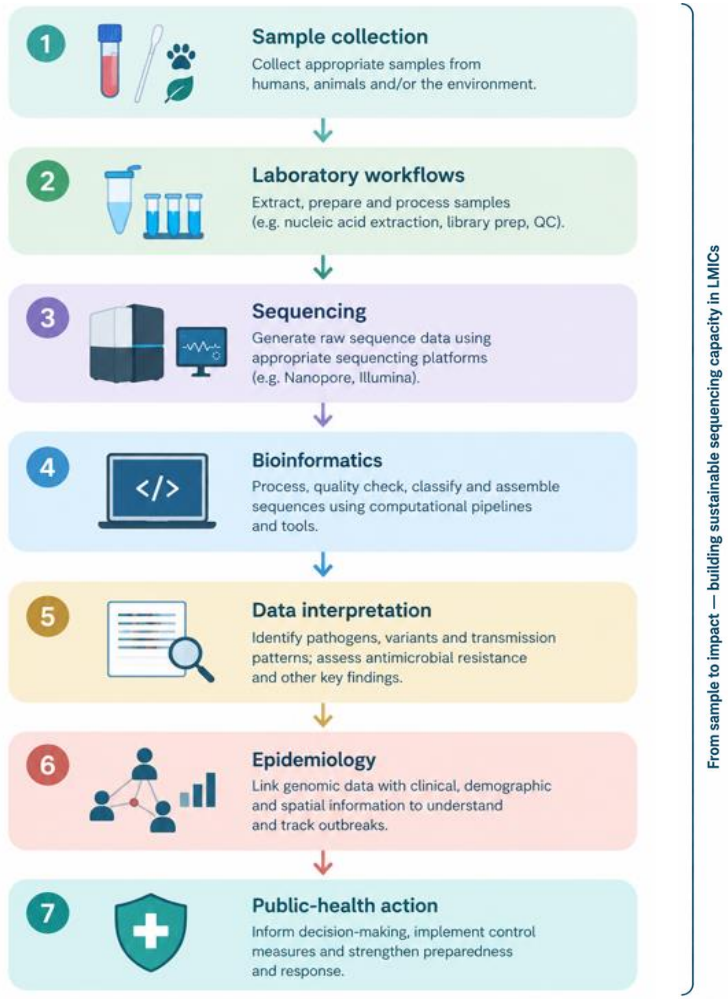
(A) SARS-CoV-2 sequences shared on GISAID per capita per continent

(B) Number of SARS-CoV-2 sequences shared per African country on GISAID

Data from July 2025

Why is WGS capacity so important in LMIC?

- Earlier local detection and characterization of (mpox) outbreaks and outbreak response
- Continued surveillance of endemic and emerging pathogens
- Better representative genomic surveillance
- One health prospective
- Reduced dependence on external laboratories
- Sustainable expertise and infrastructure



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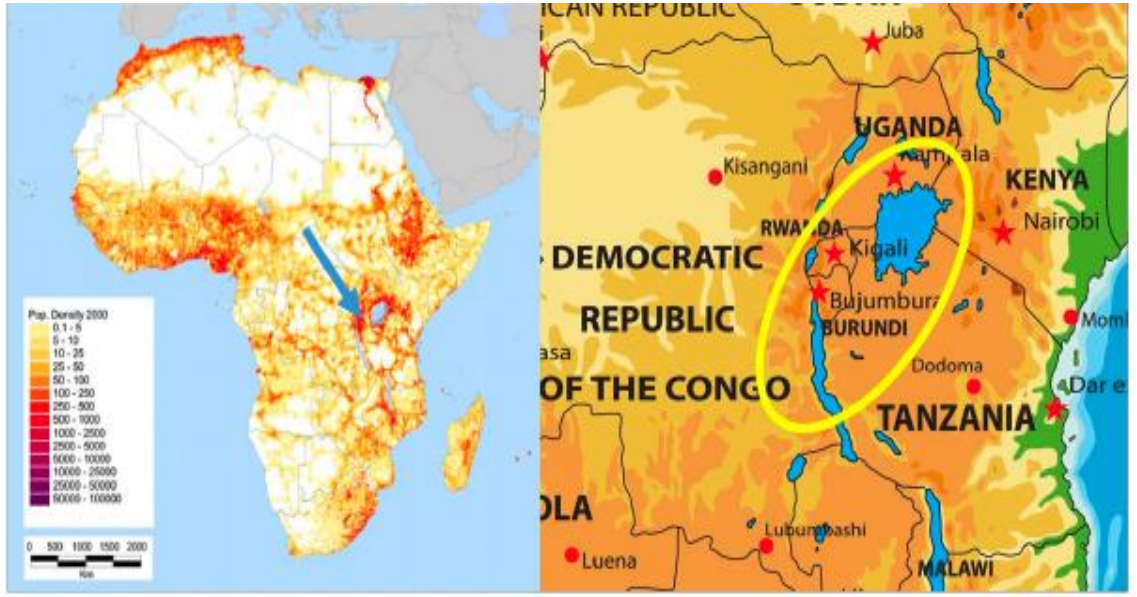


What kind of challenges do you think you would face when performing fieldwork in LMIC?

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Study Area





Central part of the African Great Lakes: a region of high population density and biodiversity, as well as huge endemic and epidemic disease challenges, yet limited surveillance

Rough and remote terrain has potential implications:

- Limited sample cold-chains leading to reduced sequencing succuss
- Limited supply-chains leading to long waiting times, high shipping costs and interruptions in supply

Timeline

2024 (January)
Mpox outbreak – WGS Rwanda

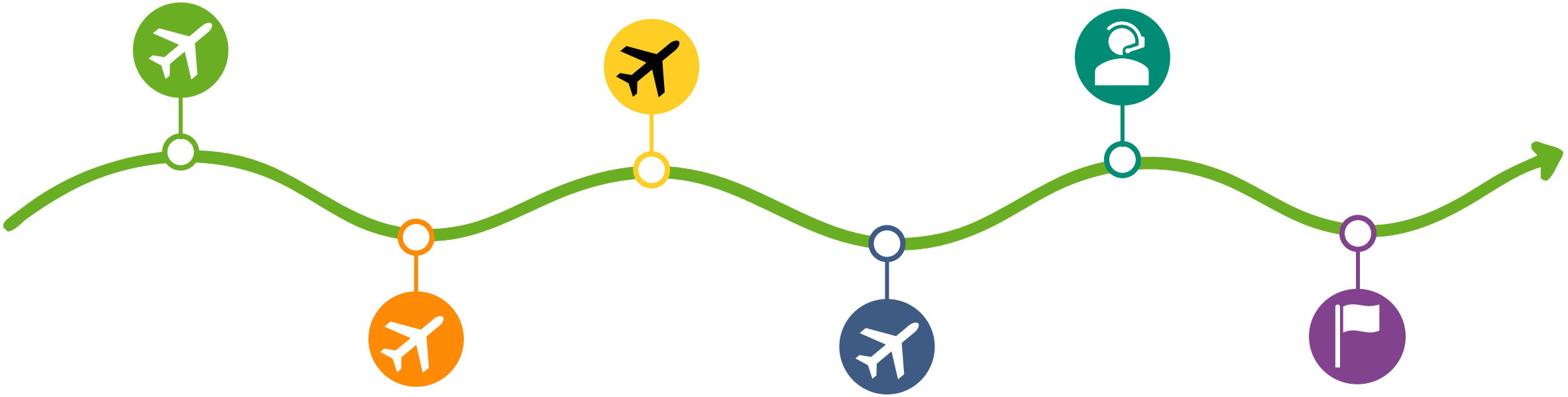
2024 (May)
Mpox outbreak – qpcr and WGS Rwanda

2024 (September)
Sequencing capacity workshop – WGS and metagenomics Tanzania

2025 (October)
Sequencing capacity workshop – WGS and metagenomics Rwanda

2026 (January -)
Remote sequencing capacity – WGS and metagenomics Burundi and Rwanda

2027…



Sequencing workshop – Rwanda (Huye) 2024 and 2025







Sequencing workshop – Tanzania (Kilimanjaro Clinical Research Institute) 2024







Metadata and sample overview

Scenario A:

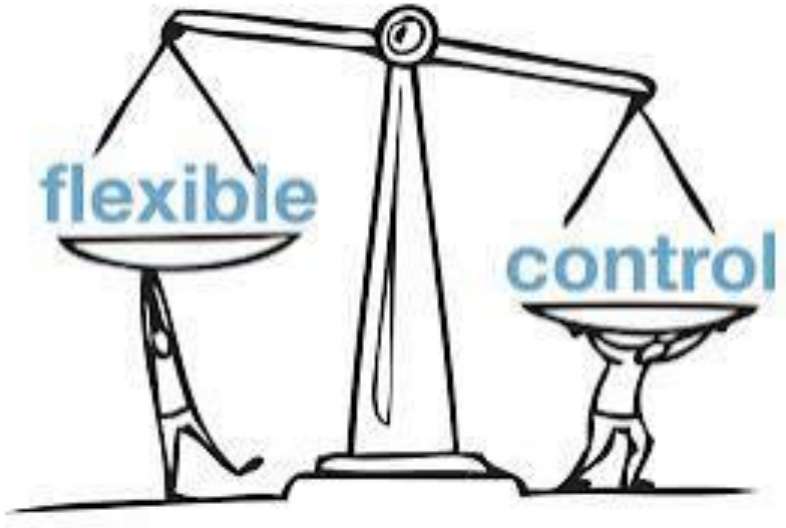
Designed samples with made up scenarios

- Best and most predictable option for a completed and rounded training (exercise) but not always reflecting conditions/challenges of partner sites
- More standardized

Scenario B:

Samples and metadata provided from partners

- Reflects challenges on sites best (wet & dry lab) but predictability is low
- More flexible → adapt to the situation



Some important considerations:

- What is the question or priority (etc. training, specific surveillance or outbreak scenario)
- Crucial to make an overview of samples, define and collect critical metadata
- Data protection measures/laws (anonymous names etc.)
- Biosafety laws, sample inactivation and transport

Planning and logistics.....





Category	Item	Remarks
Lab equipment and instruments	Real-time PCR thermal cycler *	If not present on site, inquire about availability from other departments. Pairing with back-up battery to bridge power cuts highly recommended
	MinION (Mk1B or Mk1D)	Mk1D has improved temperature regulation (useful for air-conditioned sequencing rooms and cold climates) but is more expensive (>$1000)
	Laptop with Nvidia GPU	Nvidia graphics card with 8Gb VRAM and CUDA support, high accuracy basecalling
	Flow cells (R10.4.1)	Transport as carry-on luggage only. Do not check into the aircraft hold, as temperatures can drop below +2 °C.
	Qubit fluorometer and tubes	
	Magnetic stand	For 96 well plates
	PCR plates with matching lids/covers	
	Pipettes with matching tips	Filtered tips (1–10 µl, 10–200 µl, 100–1000 µl)
	Low-binding centrifuge/reaction tubes	1.5 ml
	Centrifuge (for tubes and plates)	BeniaLab or on-site equipment
	Thermo cycler	BeniaLab or on-site equipment
	Dry heating block	BeniaLab (small volumes) or on-site equipment
	Facilities	Diagnostic laboratory with access to a biosafety cabinet/PCR cabinet with UV light. Alternatively, mobile glove box systems (e.g. EMLab), which limits throughput and increases contamination risk. Autoclave for solid and liquid waste disposal
	Personal protective equipment	Ideally on site. Lab coats, N95 masks, disposable laboratory gloves, safety glasses
	Staff requirements	Trained personnel (sampling, handling, pipetting, biosafety regulations, etc.)
Cold chain: consumables	QS Hot Start polymerase/buffer **	Platinum™ SuperFi DNA Polymerase (Invitrogen) **
	Ultra II End-prep enzyme mix/buffer **	Taq DNA Polymerase recombinant (Thermo Scientific), (Invitrogen) and T4 Polynucleotide Kinase (NEB) T4 Polymerase (NEB) **
	Blunt/TA Ligase Master Mix **	T4 DNA Ligase (NEB) **
	Quick T4 DNA Ligase/buffer **	T4 DNA Ligase (NEB) **
	Qubit dsDNA HS assay	Broad range assay also an option
	dNTP mix	10 mM each
	Real-time PCR primers and probes	Ideally lyophilized, protect probe from light
	1x TaqMan Universal PCR master mix	For DNA viruses
	Native Barcoding Kit 24 V14	Includes SPRI beads (AMPure XP) and barcodes
	Flow Cell Wash Kit and Flow Cell Priming Kit	Optional. Possibility to increase cost-efficiency of Nanopore runs with few samples. Support extra runs with washed flow cells. Halve library loading reagents for short runs/runs on washed flow cells
	MPXV amplicon assay	Amplicon size may be adjusted based on sample quality and complexity
Consumables	Nuclease-free water	
	DNA/ Total Nucleic Acid Isolation Kit	Versatile kit for microbial nucleic acids
	Ethanol (absolute) *	Ideally get on site from, for example, a pharmacy. Transport via airlines requires checks (dangerous goods, flammable materials)
	Decontaminant *	Concentrated bleach, bleach tablets, Virkon S
	Sample preservation medium	Transport medium, virus transport medium. Ideally already in use on site
	Bovine serum albumin (ultrapure)	Optional, freeze dried
Other	Positive controls *	National regulations apply, export licence required in advance
	External hard drive (encrypted)	Data backup, Anaconda (data science toolkit), software, databases
	Safety data sheets for reagents	For import - transport
	Donation letter	List of items including manufacturing date, lot numbers and expiration date
	Invitation letter	Hosting institute/collaborator with contact information
	Visa	Respective government department
	Medical prophylaxis	Vaccinations, malaria prophylaxis
	Printouts/manuals	Protocols and manuals in multiple languages (English + local language(s) where applicable)
	Mobile phone with eSIM support	Internet necessary to start MinION runs, Offline Nanopore runs must be organized in advance (contact regional representative). Phones are often preferred over email for communication.
Bioinformatic toolkits and software	EPI2ME	(Z)IS-based workflows for amplicon and metagenomic data, initially requires internet to download databases and set up. Can be run offline afterwards
	CZID Labs (retrospective recommendation, planned application in future sequencing activities)	(Z)IS-based workflows primarily for viral and bacterial metagenomic samples
	WSL 2	Linux for windows laptops (WSL 2)
	Anaconda	Linux-based bioinformatics software management framework
	UGENE	Software to inspect BAM files
	AliView	Software to inspect multiple sequence alignments
	Squirrel	Alignment and phylogenetics mpox virus pipeline
	Nextclade	Nextclade web is convenient and requires little traffic
	Reference databases	RefSeq sequences for consensus generation and MPXV background sequences for phylogenetic analysis

Scheule el al., 2025

What is currently available on-site?

What do we need to send?

- Storage and transport conditions (e.g., cold chain differences)
- Permission forms
- Transport challenges (e.g., boxes stuck at airport security)

What do we need to bring in-person?

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Sequencing assay depend on sample quality

Question: Can you think of some strategies to retain sample quality?

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Sample storage, quality and transport

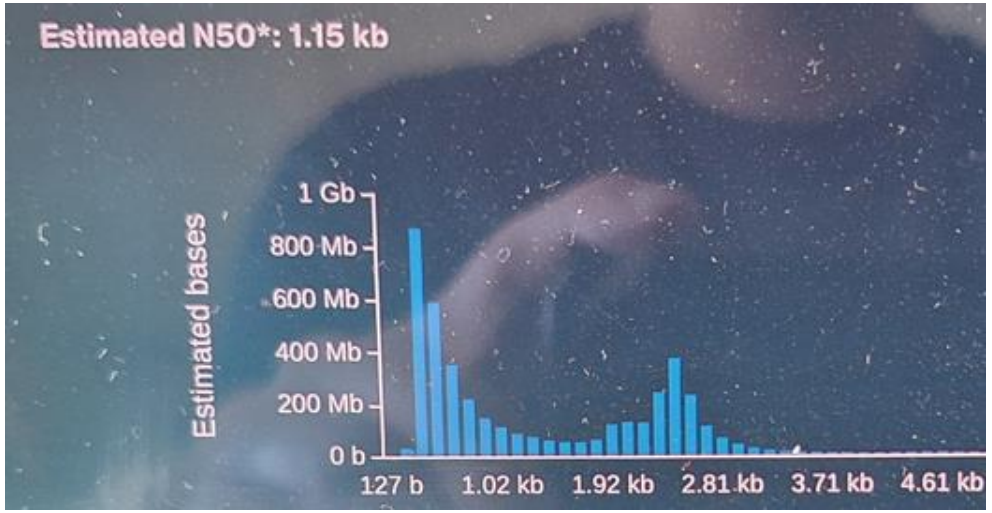
Question: Can you think of some strategies to retain sample quality?

- Cold chains on site, storage capacity (requires +4 °C fridge and -20 °C freezer)
- Sample preservation medium (e.g. Virus transport medium)
- Extract nucleic acids before (long term) storage/transport
- Wet lab strategies (e.g. centrifugation) and dry lab strategies (e.g. designing short amplicons sizes) can help with potentially degraded and low viral load samples

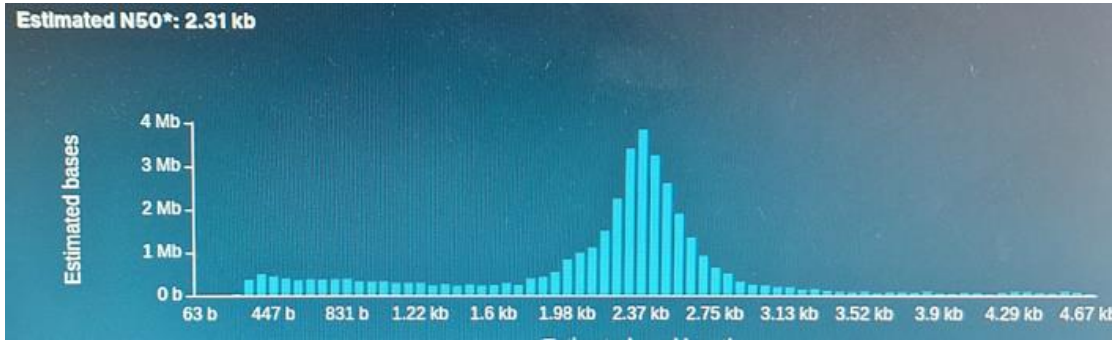
Sample storage, quality and transport

WGS (and metagenomics) assays reliant on sample quality

2 days transport with failed cold chain and poor sequencing result



Mpox EQA sample (same reagents)



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NGS/qPCR very prone to pipetting inaccuracies and cross contamination

Can you think of some ways to reduce the impact ?

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Equipment on site and cross contamination risks

Question: Ways to reduce the impact ?

- UV light (if not available, DNA buster/chlorine wipes etc.)
- Changing gloves often (if possible)
- Increase pipetting volumes above 1µl
- Filtered pipette tips and compatible tips/pipettes
- Negative controls (extraction control and non-template controls)!

Equipment on site and cross contamination risks

NGS/qPCR very prone to pipetting inaccuracies and cross contamination

Try to separate extraction and amplification steps as much as possible



Pipette and tips mix



Power cuts and internet

Occasional power cuts can impact assays all the way from nucleic acid extraction to data analysis. Internet is also initially required to start nanopore sequencing.

Do not edit
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Can you think of some ways to best negate power cuts and internet challenges?

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Power source and internet

Question: Strategies to best negate this?

- **Battery/power backup**

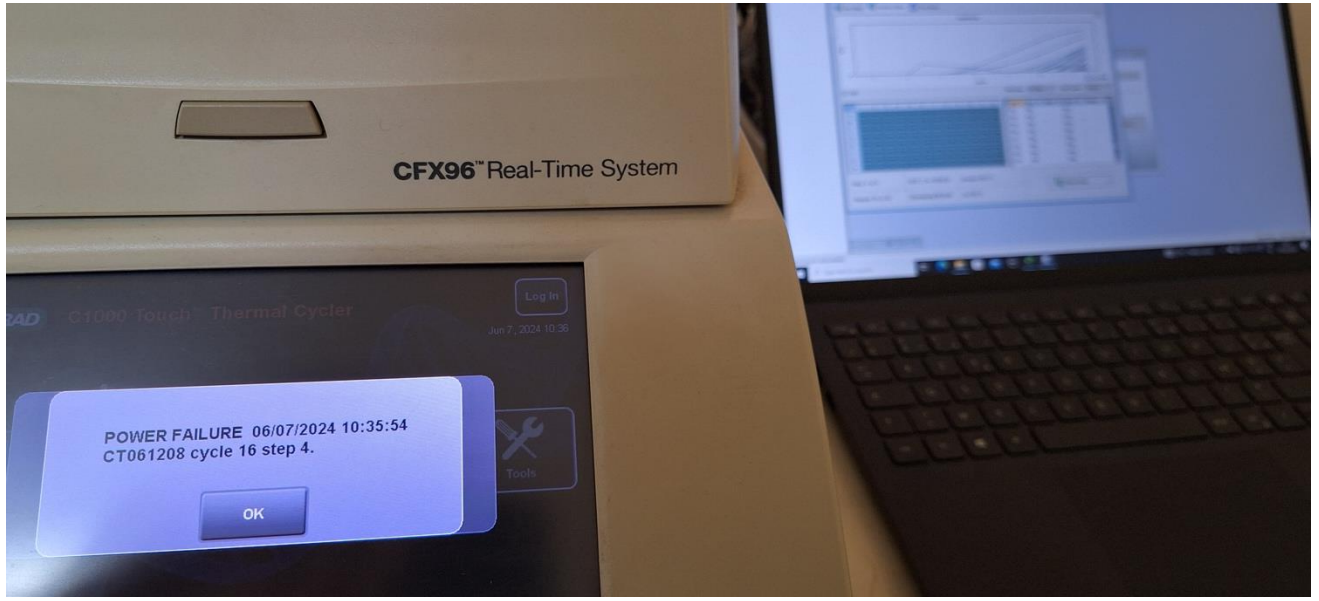
→ Essential on the devices prone to power cuts (real-time PCR, POC etc.)

- Laptops can bridge power cuts during nanopore sequencing and data analysis pipelines
- E-sims or buying local sims for back-up internet

Power source and internet

Occasional power cuts can impact assays from nucleic acid extraction all the way to data analysis. Internet is also initially required to start Nanopore sequencing.

Two power cuts during orthopox PCR



Bioinformatic challenges within LMIC

- People have laptops to analyze with different specs (database sizes etc.)
- Internet speed might be limited
- Backup data on hard drive (also good for distributing programs, databases etc.)
- Balancing time between wet and dry lab
- Complex data interpretation



In summary

- LMICs remain disproportionately affected by infectious disease risks
- Regional collaborative capacity building efforts are beneficial to expand genomic surveillance accessibility in these settings.
- Recognizing current capacity differences and highlighting the challenges in LMICs is crucial
  - to help expand and sustain genomic surveillance, thereby strengthening national, regional and global decision-making.
- Beyond the technical hurdles, this work has yielded over 12 peer-reviewed papers driven by interesting projects and incredible local collaborations

Recommended material to explore further

Am J Trop Med Hyg. 2024 Jan 23;110(3):417-420. doi: 10.4269/ajtmh.23-0675. Print 2024 Mar 6.

Building Research Capacity in Low- and Middle-Income Countries and Pandemic Preparedness: Lessons Learned and Future Directions

Peter H Kilmarx [1], Karen A Goraleski [2], Erum Khan [3], John F Lindo [4], Nancy Gore Saravia [5 6]

Affiliations + expand

PMID: 38266289 PMCID: PMC10919184 DOI: 10.4269/ajtmh.23-0675

Trop Med Infect Dis. 2025 Mar 31;10(4):90. doi: 10.3390/tropicalmed10040090.

Building Pathogen Genomic Sequencing Capacity in Africa: Centre for Epidemic Response and Innovation Fellowship

Eric Agboli [1 2], Molalegne Bitew [3], Christian N Malaka [4], Tiangay M P S Kallon [5], Alhaji M S Jalloh [5], Baron Yankonde [6], Doreen M Shempela [6], Jay F M Sikalima [6], Mutale Joseph [7], Mpanga Kasonde [7], Feleke M Demeke [8], Ayemfouo F I Valdese [9], Lele B Grace [9], Godwe Célestin [10], Ana Papkiauri [11], Sado Y F Berlange [12], Janet Majanja [13], Vane K Omwenga [13], Evalyne N Wambugu [14], Samuel M Kariuki [15], Alex A Mwanyongo [16], Ubheeram Jaykissen [17], Caroline Abanto Alvarez [18], Seyni Ndiaye [19], Benjamin Moswane [20], Ester K Adamson [21], Mariam Makange [21], Luka Sote [22], Ibrahimm Mugerwa [23], Julius Sseruyange [24], Patrick Semanda [24], Brian A Kagurusi [24], Abdualmoniem O Musa [25], Kourush Fassihi [26], Lavanya Singh [26], Monika Moir [27]

Affiliations + expand

PMID: 40278763 PMCID: PMC12030795 DOI: 10.3390/tropicalmed10040090

communications medicine

A Nature Portfolio journal

Perspective

https://doi.org/10.1038/s43856-026-01713-4

Capacity building for genomic surveillance of mpox and other emerging diseases in resource-limited settings within the African Great Lakes region

Check for updates

Leonard Schuele [1], Néhémie Nzoyikorera [2,12], Jean Claude Udahemuka [3,4,12], Hayley Cassidy [1,12], Leandre Murhula Masirika [5,6,7,12], David F. Nieuwenhuijse [1], Cassien Nduwimana [2], Richard Molenkamp [1], Joseph Nyandwi [2], Marjan Boter [1], Jules Minega Ndoli [8], Luca M. Zaeck [1], Jean Pierre Musabyimana [3], Rory D. de Vries [1], Justin Bengehya Mbiribindi [9], Freddy Belesi Siangoli [9], Saria Otani [10], Frank M. Aarestrup [10], Marion Koopmans [1], Pacifique Ndishimye [3,11,12] ✉ & Bas B. Oude Munnink [1,12] ✉

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- Marjan Boter
- Luca M. Zaeck
- Justin Bengehya Mbiribindi
- Freddy Belesi Siangoli
- Saria Otani
- Evodie Uwibambe
- Saria Otani
- David Nieuwenhuijse
- Frederik Duus Møller
- Jean Pierre Musabyimana
- Frank Aarestrup
- Fabrice Ndayisenga
- Pacifique Ndishimye
- Boaz Wadugu
- Marion P.G. Koopmans
- Bas B. Oude Munnink











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