Genomics training in the EMRO region

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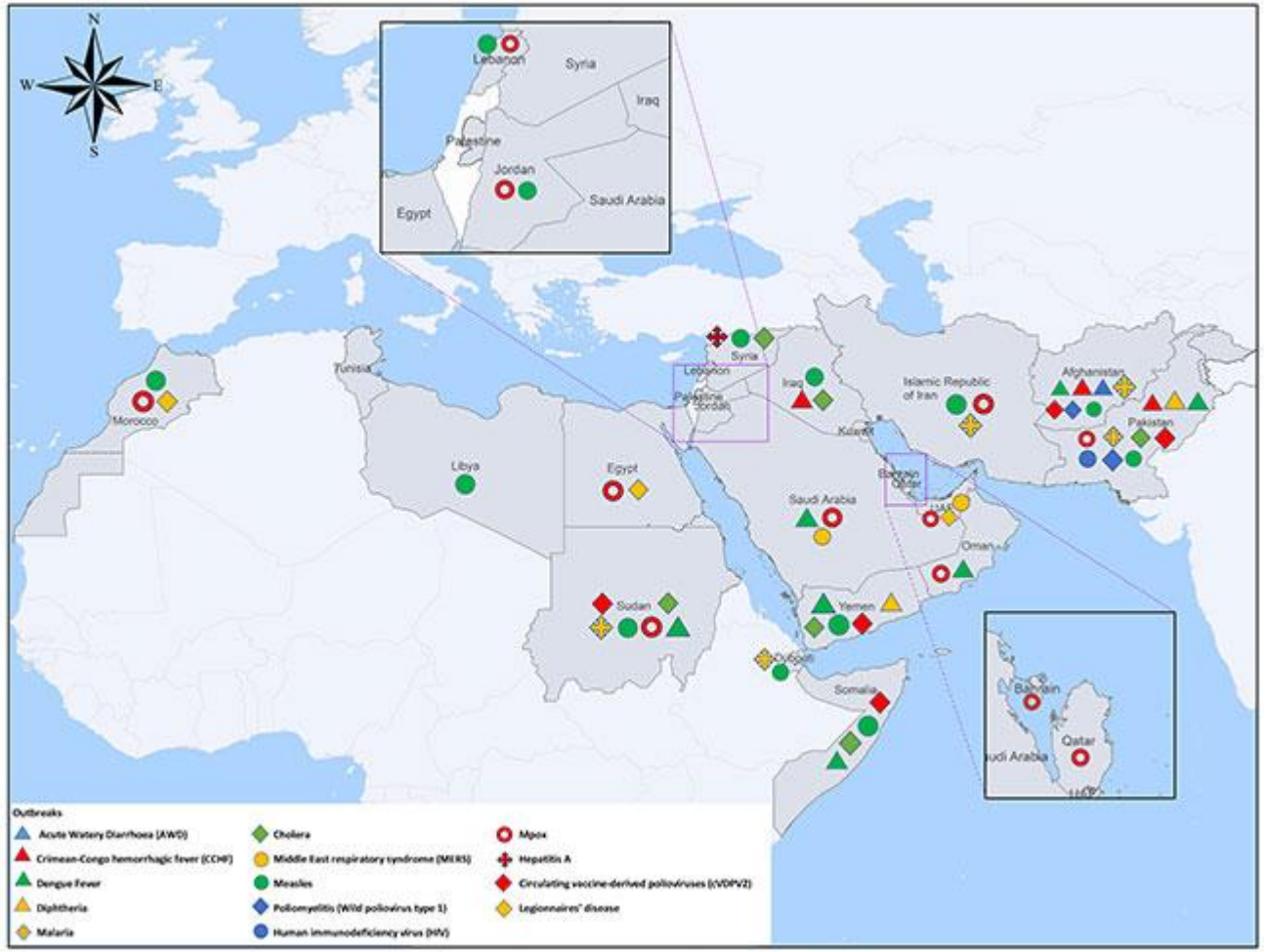
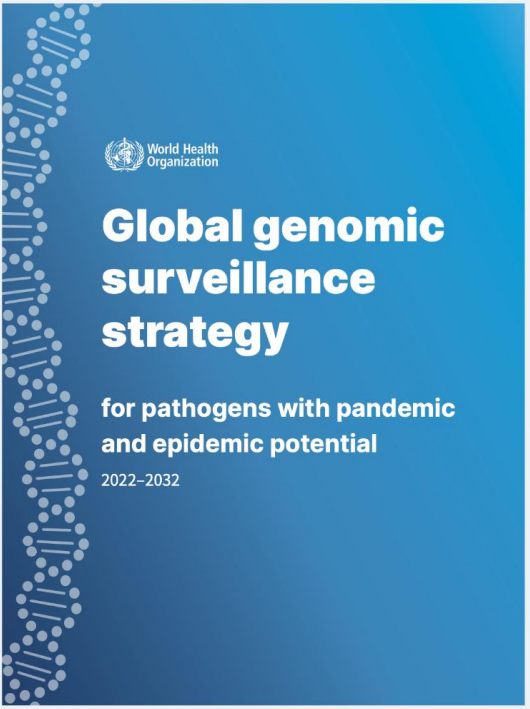
WHO EMRO WHE/IHP Lab Team

EMRO: Extensive Health Challenges



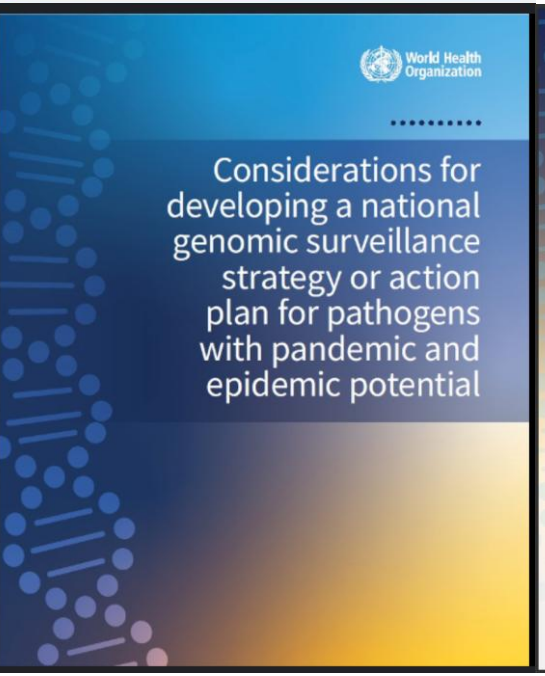
https://www.emro.who.int/pandemic-epidemic-diseases/outbreaks/index.html

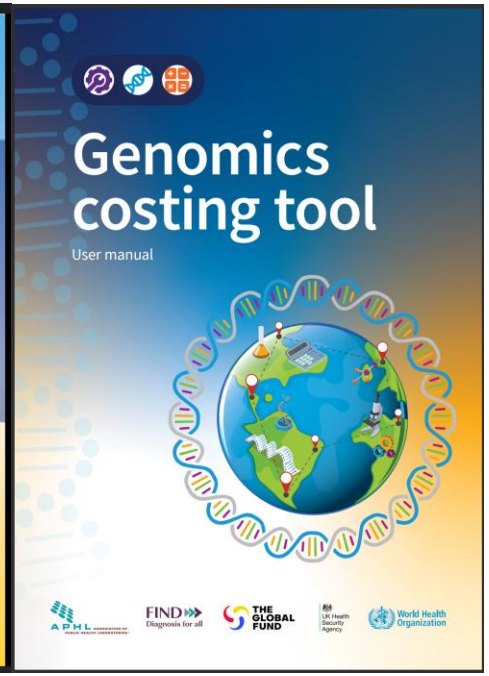
- Geographically expansive, with diverse climates
- Diverse populations
- 55 ongoing outbreaks
- 11 complex emergency countries
- Global warming impacting weather patterns.
- Wide range of technical, logistical and laboratory expertise.



WHA Resolution 74.7

- *"World Health Assembly urges Member States to increase their capacity to detect new threats, including through laboratory techniques such as genomic sequencing."*





- Global genomic surveillance strategy launched in 2022.
- Costing and policy tools released in 2023.
- International Pathogen Surveillance Network (IPSN) launched in 2023.

What does genomic surveillance tangibly need to function?

Outcome	Team
Clearly defined goals for sequencing (priority pathogens etc).	Regional strategy (10 priority pathogens for genomics)
Strong logistics, infrastructure and funding.	Dubai Logistics Hub and partners
A functional surveillance and epidemiology network.	Epidemiology and RRT network
Sample collection and identification kits (and know how to use them).	Logistics, labs and training
A strong laboratory network.	Logistics, labs and training
Clearly defined diagnostic algorithms for in vitro diagnosis.	Labs and training.
Capacity to produce and analyse high quality, meaningful sequencing data.	Labs, EQA, bioinformatics, genetic epidemiology, training teams
Ability to interpret and assess genomic data in relation to public health (risk assessment).	Bioinformatics and epidemiology, public health teams.
Strong links between the laboratory, epi and public health teams (preferably OneHealth).	Communications, data and information sharing teams
Ability to communicate and share that data with partners to mount a response (or not).	Communications, data and information sharing, national and regional policy.

Jordan, 2023

Influenza and other respiratory viruses Open Access isirv

ORIGINAL ARTICLE | Open Access

Key aspects defining the development and implementation of a regional genomic surveillance strategy for the Eastern Mediterranean Region

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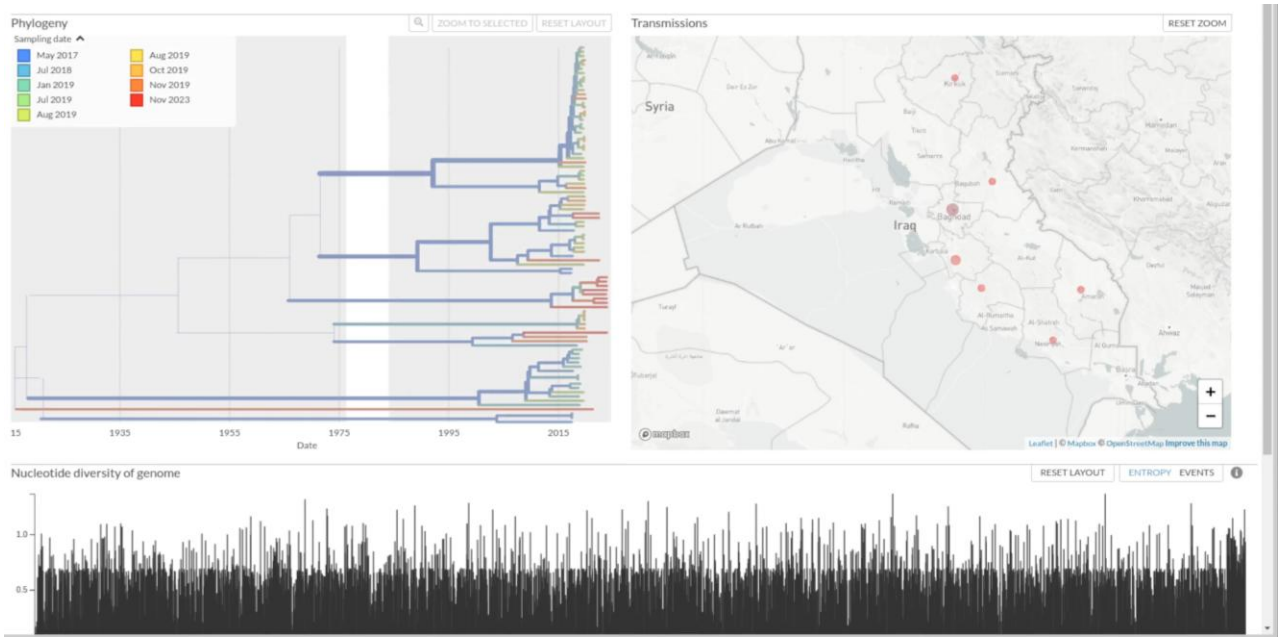
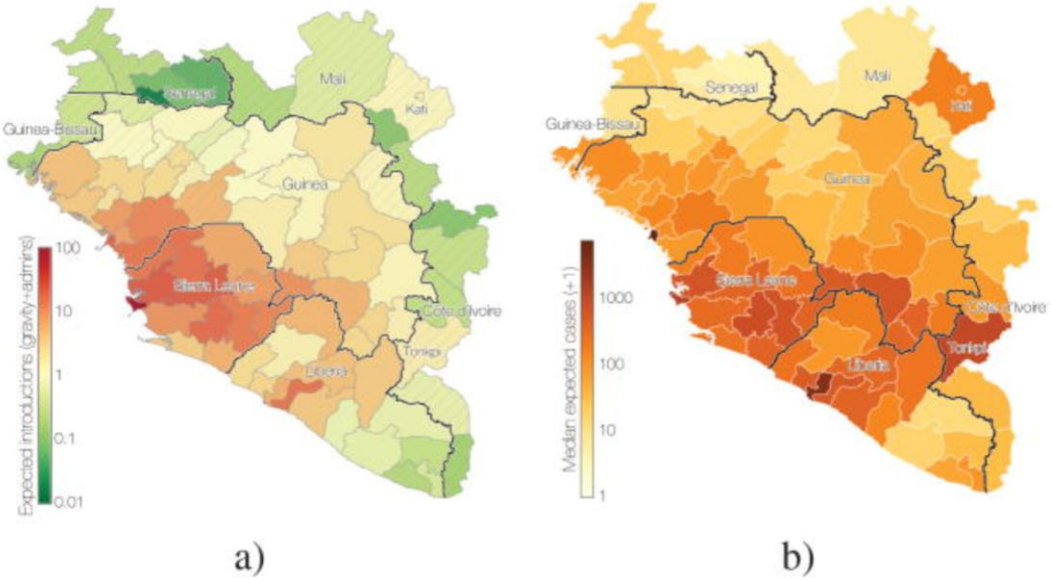
“In the face of all the challenges, why does sequencing matter?”

- **When used properly**, sequencing provides invaluable **support** for epidemiological, diagnostic and therapeutic interventions.
- Sequencing provides support for epidemiological tracing across and within borders.
- Monitoring AMR to guide drug distribution for clinical support.
- Monitor efficacy of public health interventions.
- At this stage sequencing is **NOT** a primary diagnostic tool approved for use for anything other than COVID-19 unless under extraordinary circumstances.

Transmission chains

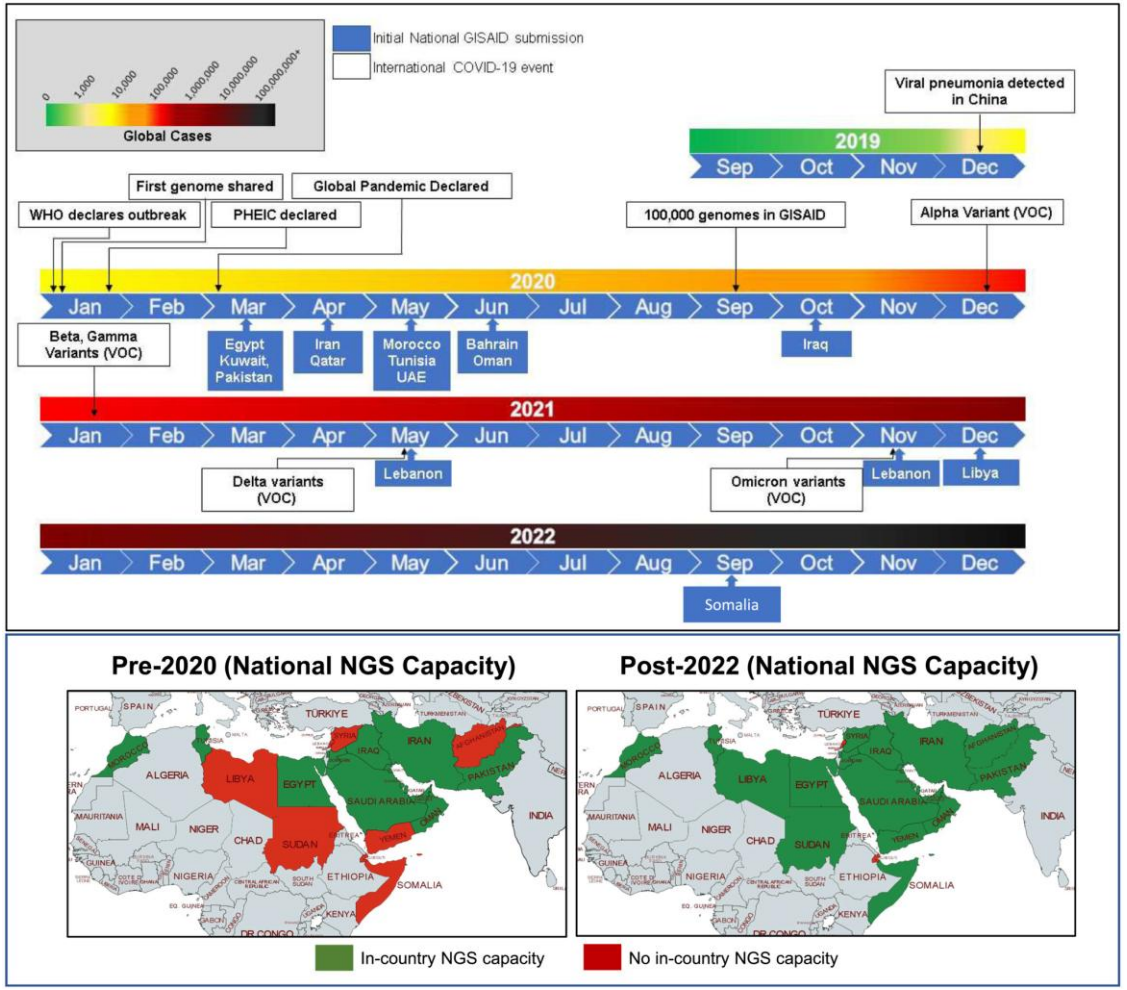


Effect of interventions



Genomic goals

- Build on and strengthen genomics capacity in the Region.
- Integrate genomic surveillance with existing surveillance networks to enhance awareness and understanding of disease spread in the region to prevent outbreaks becoming epidemics or pandemics.
- Build a sustainable regional network of genomic expertise at both the laboratory and surveillance level
- Support the development of sustainable genomic surveillance at the National policy level.



21/22 countries now have sequencing capacity at the national level for COVID-19, expand this to other viral and bacterial pathogens.

EMRO NGS Trainings 2023/4

Regional/Sub-regional trainings

- ***AWD Genomics and Bioinformatics, AUB Lebanon, 2023***
- Arboviral Genomics and Bioinformatics, CPHL Oman, 2023
- Bioinformatics, Online, CLC Workbench, 2023

National (in-country) trainings

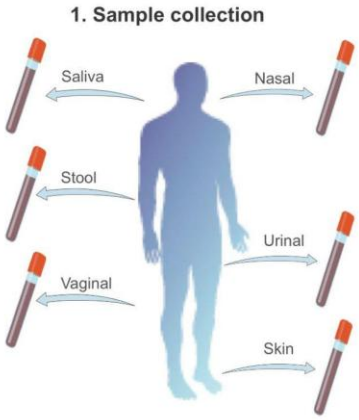
- ***AWD Genomics***
  - ***Sudan, Yemen, Iraq, UAE, Oman***
- Arboviral genomics
  - Iraq, UAE, Oman, Afghanistan*
- Respiratory genomics
  - UAE, Oman, Afghanistan*, Lebanon*

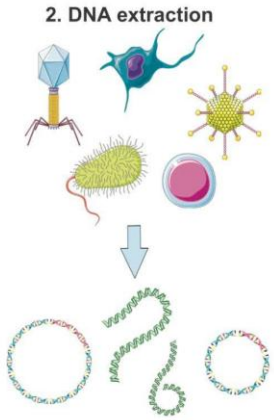
Remote Trainings / Data analysis

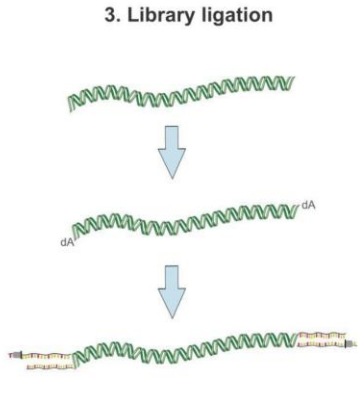
- AWD genomics
  - Iraq, UAE, Sudan, Oman

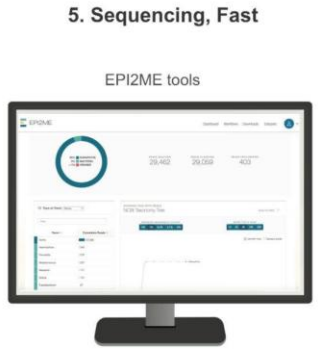
**Pending in July*

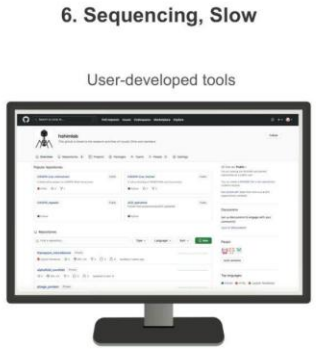
Cholera Sequencing: Nanopore rapid sequencing











- Identify lineage, AMR, strain and toxigenicity in one assay.
- One plate or broth for culture.
- 24 hours from sample receipt to sequence using ONT rapid barcoding.
- Requires an extraction kit, flowcells and a barcoding kit, plus some ethanol.
- Simple, reproducible protocol
- ~$90USD/sample (if barcoding).

Why start with Nanopore?

By 2022, 21/22 countries in the region had either a MK1C or a GridION to sequencing COVID-19.

The Midnight protocol was the most widely used protocol in the region.

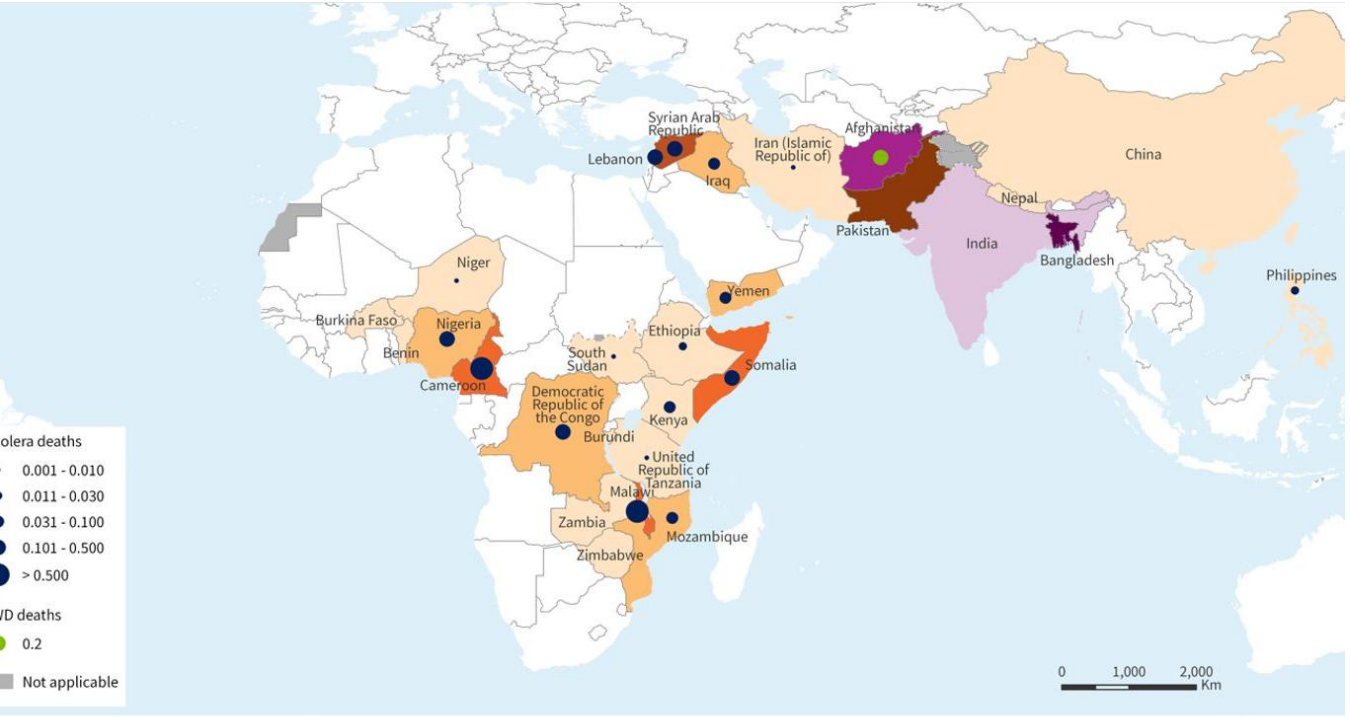
Logistically speaking, the nanopore offers the smallest footprint and ease of use in complex settings.

Adapting existing protocols to use Artic-style approaches is possible as is shotgun sequencing

https://onlinelibrary.wiley.com/doi/full/10.1111/irv.13205

What do we hope to achieve: **Cholera**

- Cholera outbreaks declared across almost the entire region.
- Vaccine resistant strains?
  Increased virulence?
  Drug resistance?
  Novel transmission chains?
  Effect of WASH/interventions?
- **NOT primary diagnosis** outside specific circumstances.



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Data Source: World Health Organization, United Nations Population Division (population prospect 2021)
Map Production: WHO Health Emergencies Programme
Map Date: 9 December 2022

World Organi

AWD Training Structure (Nanopore)

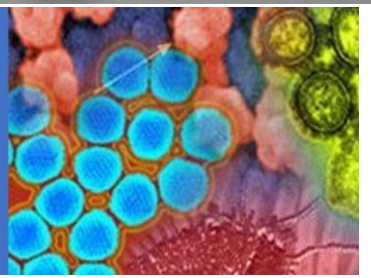
- Theory (4-10 hours)
  - Bacteriology basics.
  - NGS technology.
  - Surveillance and interpretation of sequencing data.
  - Biosafety and sample handling.
  - Data management (the importance of metadata)
  - Quality control.

Sampling and Testing Strategies:
What are they and why do we need them?

Next-Generation Sequencing:
What is it and what can we do with it?

Targeted vs Non-targeted sequencing

Shotgun Metagenomic Sequencing



Metagenomic Analysis:
Bioinformatics

On the Importance of Data Management and Quality Control

AWD Training Structure (Nanopore)

- Hands-on Laboratory (3-5 days)
  - Primarily Nanopore focused, end-to-end starting from
    sample handling through to loading and starting a sequencing run.
  - Detailed, step-by-step protocols.
  - Focus on quality and prevention of cross-contamination
  - Importance of negative controls
  - Unidirectional workflows etc

Appendix 1: Bench protocol – Cholera shotgun metagenomic sequencing protocol with rapid barcoding (SQK-RBK110.96)

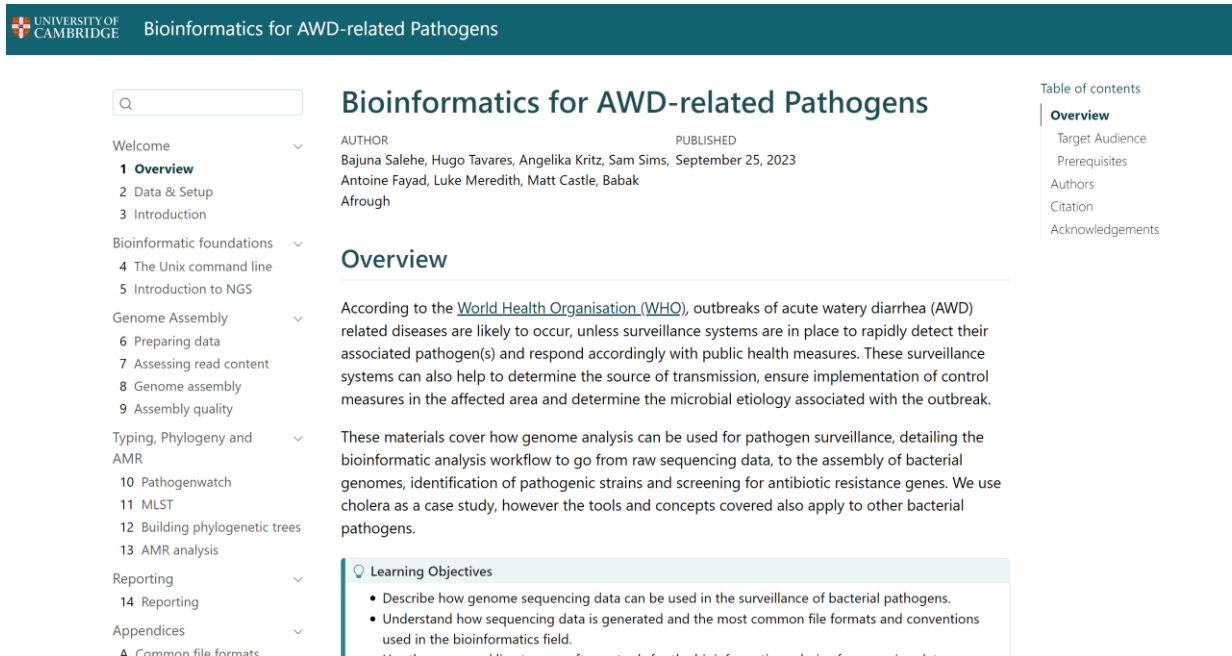
Appendix 1. Bench Protocol

Shotgun metagenomic sequencing of Cholera using Oxford Nanopore Technology and Rapid Barcoding

Date: _____
No. of Samples: _____
Operator:

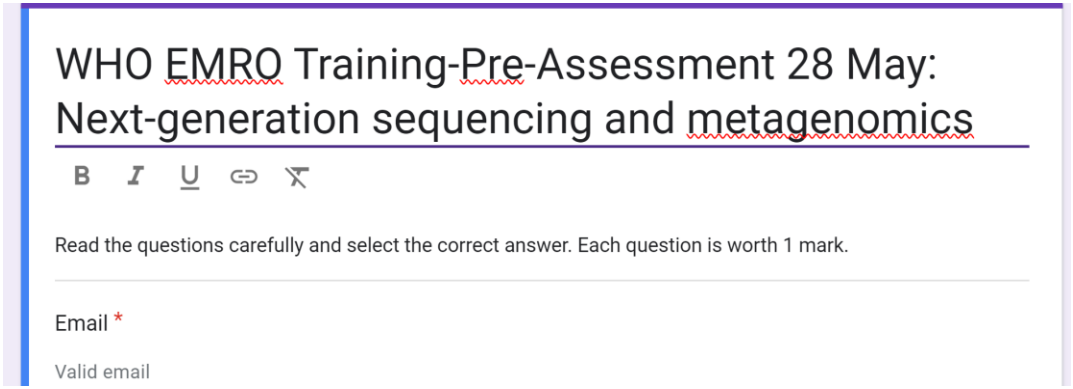
AWD Training Structure (Nanopore)

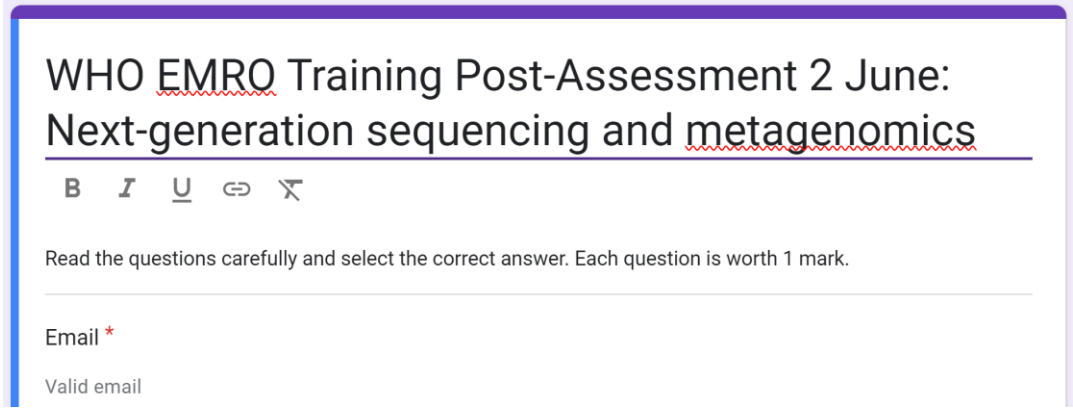
- Data analysis (2-4 days)
  - Basecalling and assembling a genome
  - Quality control
  - Basic phylogenetics (where appropriate)
  - All protocols available online
  - Support given remotely on request

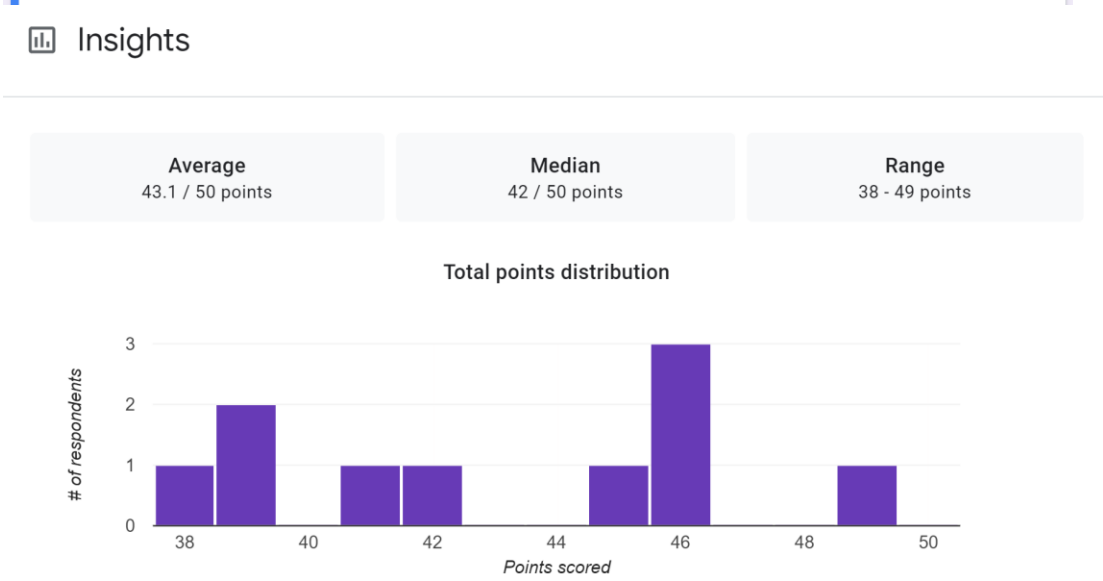


AWD Training Structure (Nanopore)

- Assessment
  - Pre- and post-course assessment of learning.
  - Look for an improvement in knowledge.
  - Scores confidential and shared only with participants.
  - Provide feedback on the key areas that need focusing on.
  - No “pass/fail” purely informative.







AWD Training Structure (Nanopore)

- Post-course feedback
  - Survey on the quality of the training and the implementation of genomics when the participants get back to their own lab.

Course Feedback - Overview

- 12 participants provided feedback in the post-course survey
- The overall feedback regarding the training was **extremely positive**

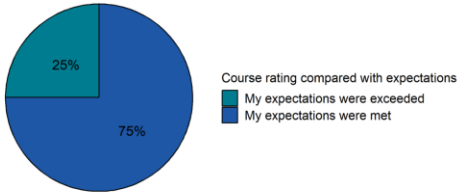


Figure 1. Participant responses to the "How did this course compare to your expectations?" question in the post-course survey. Most respondents noted that their expectations were either met or exceeded. No respondents noted that their expectations were not met.

Knowledge Increase

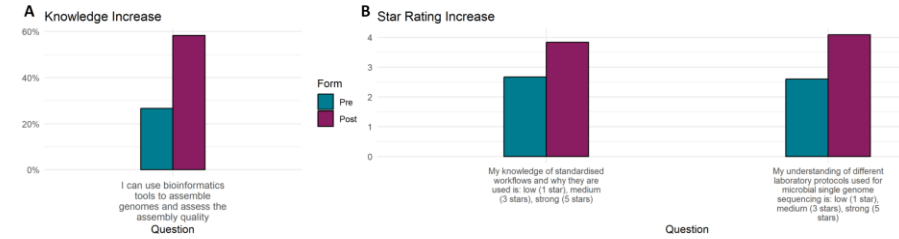


Figure 2. **(A)** Percentage of participants agreeing with statements and **(B)** mean star rating in the pre- (n = 15) and post-course (n = 12) surveys.

ens of Interest

Figure 4. When asked "What other pathogens would your laboratory be interested in receiving bioinformatics training for in the future?", the most common response was polio.

Challenges

- Availability of sequencing reagents
- Availability of sequencing training and troubleshooting (outside workshop)
- Access to Unix environment (either dedicated PC or VM)
- Sequencing errors with ONT (older chemistries)
- Steep learning curve/no prior exposure to command line

Course Delivery

- Most participants noted that the course was **too short**
  - Of these respondents, most would like to spend more time on the bioinformatics portion of the course (9/10)
  - The remainder wanted to spend more time on both the lab and bioinformatics portions of the course

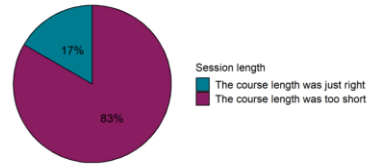


Figure 3. When asked "Do you think that the length of the course was suitable?", most participants noted that the course was too short.

Regional and National Training courses

- Series of trainings supported by the Rockefeller Foundation and WHO country offices.
- Follow-up regional trainings with National training in active outbreaks.
  - Sudan, Yemen, Oman, UAE and Iraq, Somalia (pending).
- “Train the trainers” approach, Central labs followed by sub-national.
- Focus on high-quality, reproducibility and utility of the data.

Successes

Sudan:

- Submission of 31 genomes to SRA (in progress).
- Lab work done by national staff (analysis by EMRO and NVAP).
- AMR analysis with the intended goal of supporting therapeutics.
- Toxigenicity and AMR profiles from regions.

Yemen, Oman

- Analysis of ~30 samples from across the countries (analysis ongoing) to understand where they came from.

Lebanon

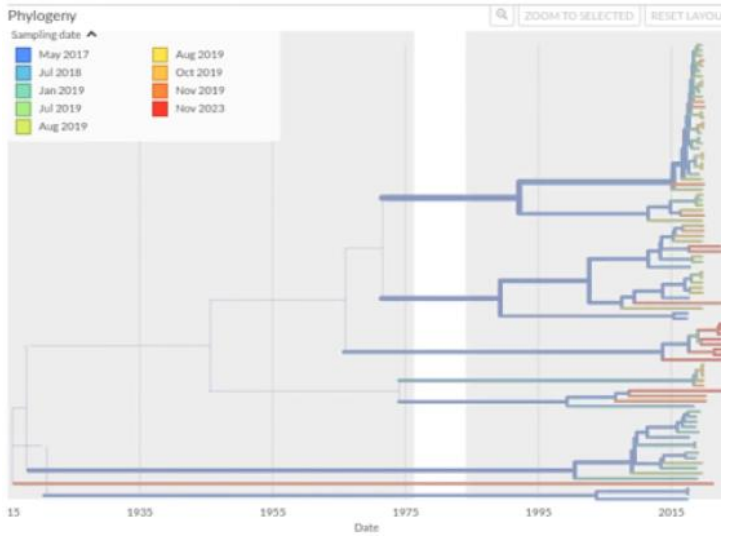
- Experience as trainers for genomics.
- Preparation of training course for Somalia.

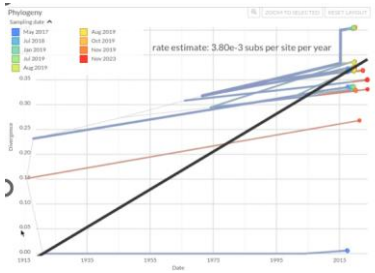
Regional

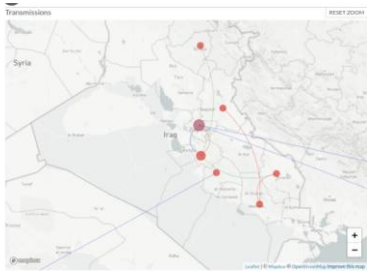
- Increase expertise in bacterial whole genome sequencing.
- Local Nextstrain monitoring for cholera transmission (in development).
- Fantastic resource for training
- https://cambiotraining.github.io/awd-pathogen-bioinformatics/

Challenges: Bioinformatics and data analysis

- The pipeline is straightforward but requires working knowledge of linux, which is a challenge.
- Understanding what the data means and how to use it.
- Phylogenetics require significant knowledge and computational power.
- Reporting in a useful manner to surveillance and public health teams is challenging.
- Minimal metadata requirements need to be coordinated from response to laboratory.







Quality Control

- While the sequencing works great, it will only correctly identify cholera if cholera samples are added.
- Sample selection is critical to making sequencing worthwhile.
- Bacteriology skills need to be excellent.
- Confirmed diagnosis is critical to cost-effectiveness and utility.

Training	Genome Coverage	Cholera Sequence Positive	Pathogens Detected
Regional (1)*	~93%	1/30	*Aeromonas, Shigella, Brucellosis, E.coli, Enterobacter, Klebsiella, Kluyveria* and more
National (1)	~90%	12/15	
National (2)	~88%	2/30	
National (3)	~94%	2/31	

Sharing and Reporting of Data

- Reporting results and sharing data with national, cross-border, regional or international partners to allow for public health discussions to be had.
- Doing this is such a way that it's non-punitive.

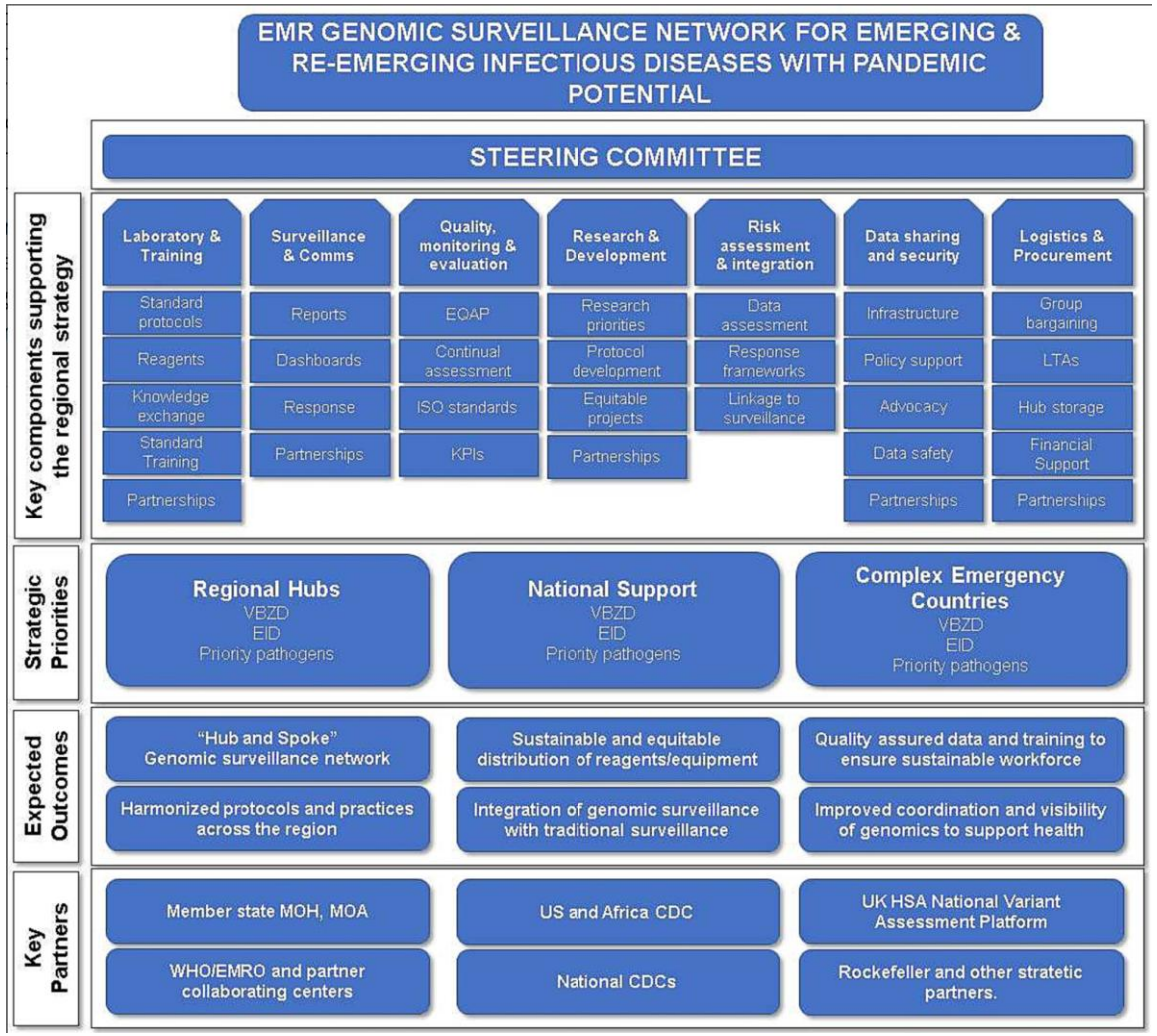
Frustration

Regional Challenges

Logistics and costs	Endless problems getting reagents to the EMR, particularly conflict zones and even hub locations.
Expertise and bioinformatics	Laboratory training is 1/3 of the total story, ongoing training needed for bioinformatics and data analysis.
	Machine expertise…every single MK1C has broken down in the past 12 months, and needed remote repair!
Data sharing and interpretation	Limited willingness to share data outside the region due to previous experiences with partners.
Responsible and useful use of data	Understanding what the data actually shows and responding accordingly.
Funding and Willingness	International funding for preparedness is limited, so needs to be used to ensure the maximal return on investment.

Tackling the challenges

- Regional strategy for genomic surveillance.
  - Steering committee and technical working groups.
  - Continued collaboration with GTFCC.
- Continued support the development of national genomic surveillance strategies.
- Continued support with access to funding and sustainable implementation of genomics.
- Continued advocacy for sharing of data and equitable sharing of reagents/expertise across the north/sound divide.
- Support with the implementation of genomics for clinical benefit.
- Improving our relationship with suppliers and funding bodies.



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