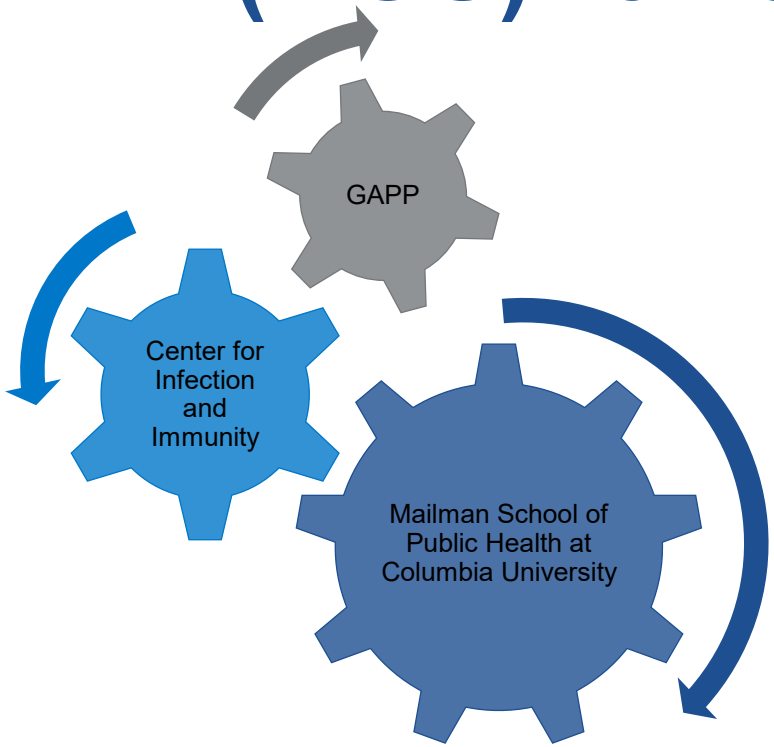
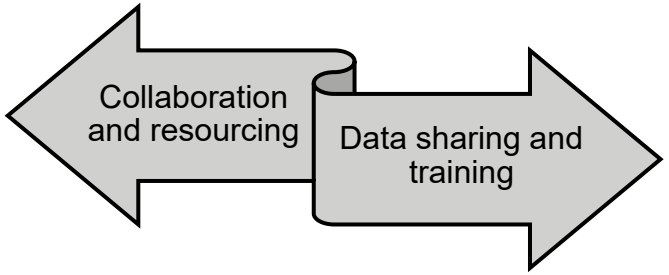
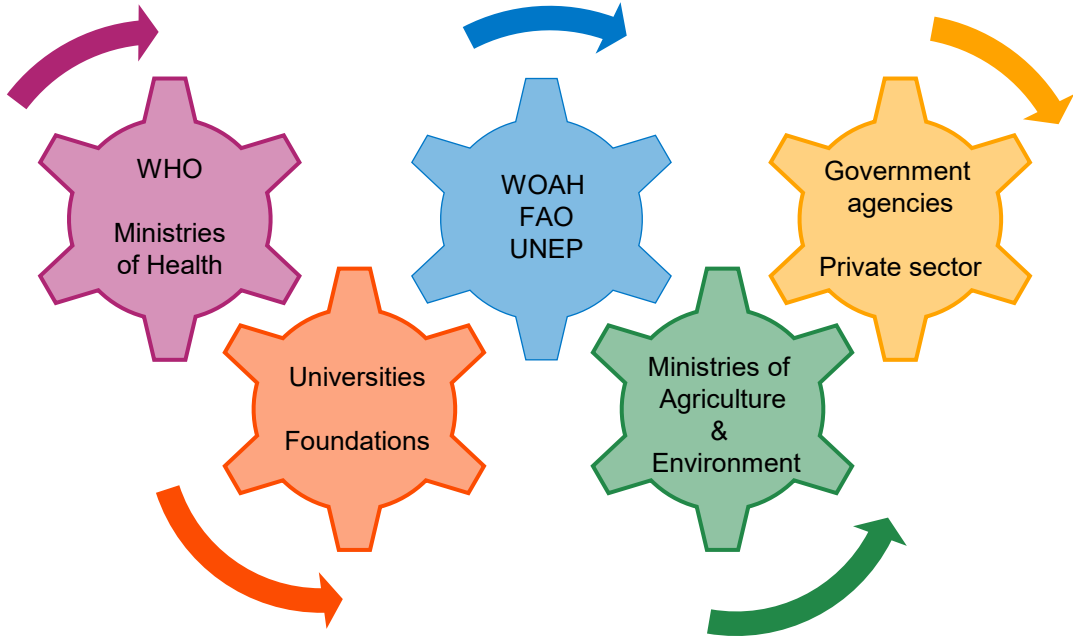
Optimizing use of next generation sequencing (NGS) for One Health biothreat reduction







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Steps of FMD detection and response

Day	
0	Alert
1	Deploy to field to take sample
2	Sample to lab
3	PCR for FMD (and maybe other differentials)
4	Serotyping – could fail due to insufficient sample volume
5-??	Ship to reference lab
??	Genotyping for molecular epidemiology

Future state

Day	
0	Alert
1	Deploy to field to take sample
2	Sample to lab
3	PCR for FMD (and maybe other differentials) + genomic data (serotype, epidemiology, attribution)
4	Serotyping – could fail due to insufficient sample volume
5-??	Ship to reference lab
??	Genotyping for molecular epidemiology

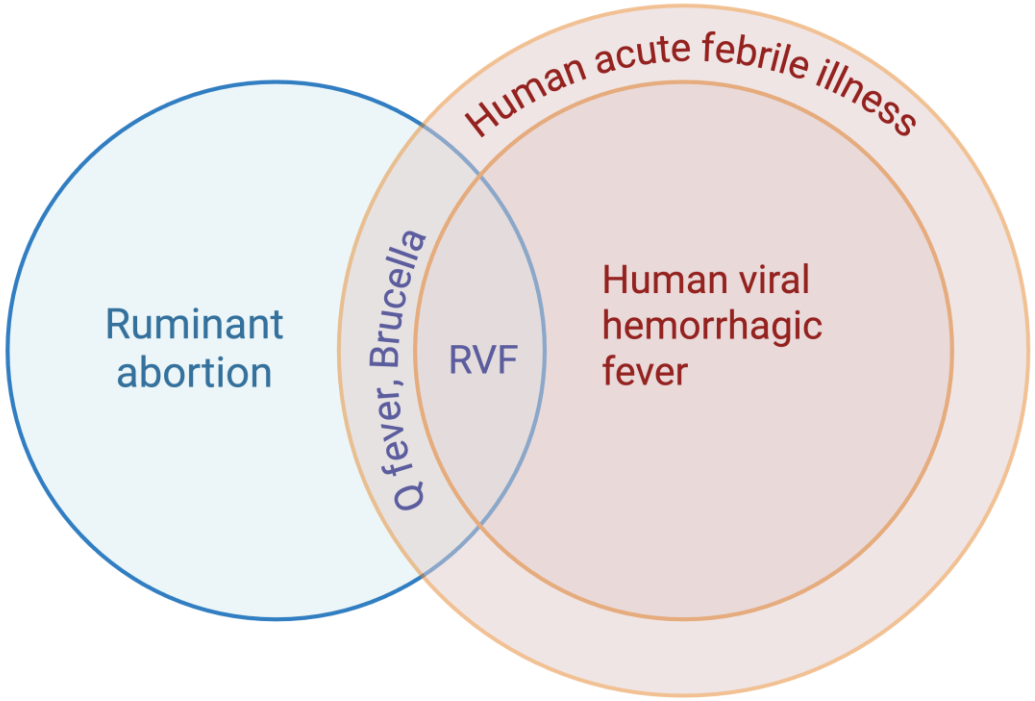
Key messages

- Consolidate genomics capacity within the member state in a One Health fashion
- Optimize the veterinary diagnostic network
- Select a cost-effective genomics method
- Ensure access to genomics for genomic forensics within the member state

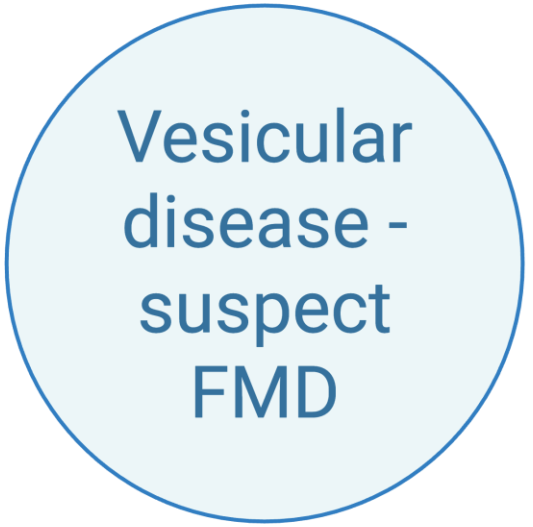
Sustainability – One Health Paradigm

- Shared resources (consolidated genomics capacity)
  - Substantial NGS capacity exists within human clinical medicine and public health systems
  - Demand for services is making that sustainable
  - Integrated surveillance including integrated reference laboratory capacity is possible
    - the veterinary sector should seize the opportunity to integrate as well
  - To enable and assure cost effective surveillance, including for non-zoonotic disease in the context of food security and trade, the veterinary sector should aggressively pursue selective use of NGS, in a One Health approach

Sometimes the entire surveillance and lab system can be harmonized



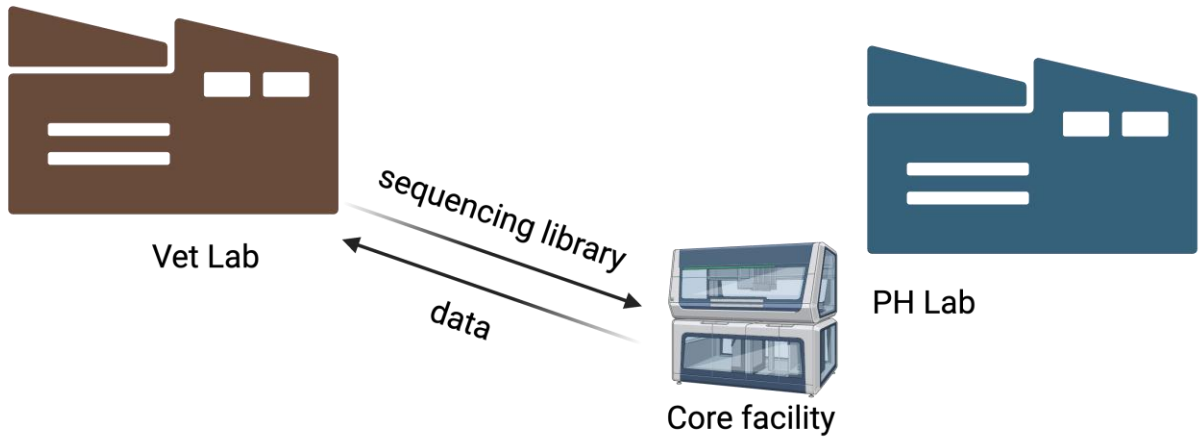
Sometimes it must be separate



But genomics is genomics if you use the right method

Where to do the testing – barriers to consolidation

- Connect the optimized diagnostic network and outbreak response system to one national reference genomics center
- Also enables direct molecular monitoring of high containment labs if present
- Need to have agreements to run extracted materials from animal samples in public health and human clinical labs



Library prep off site, then sequence in a central (“core”) facility, physically remove data off of the machine, analyze off site

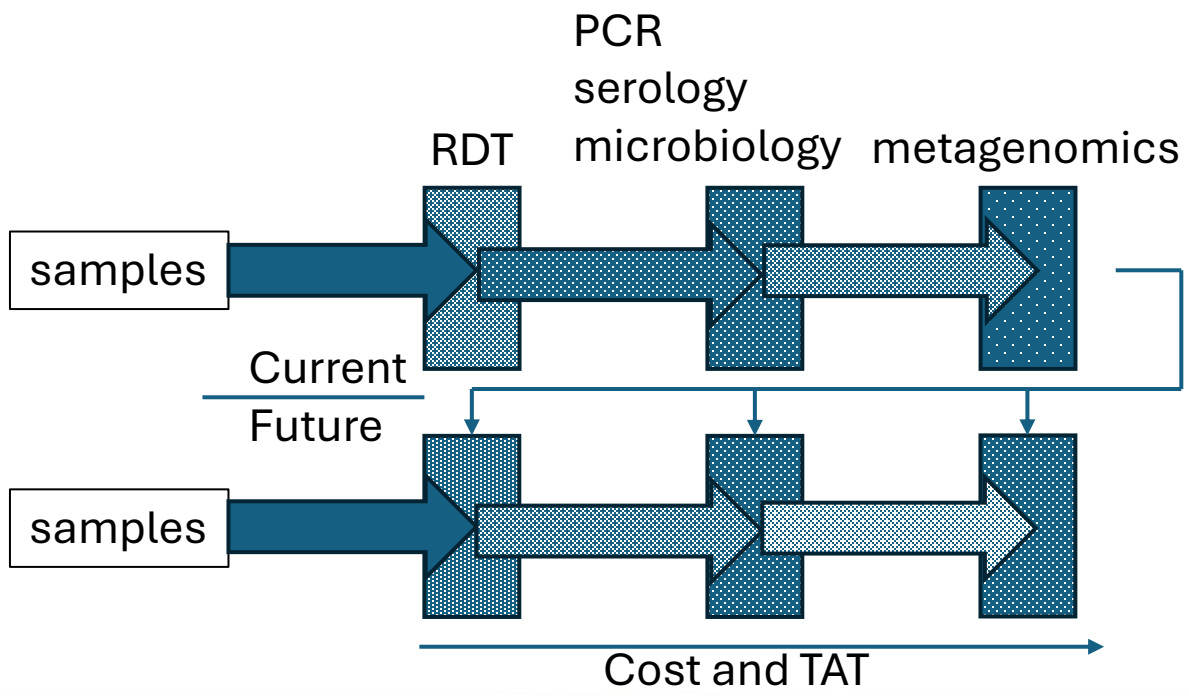
Sustainability – Diagnostic Network Optimization (DNO) and method selection

- Optimize routine diagnostic networks
  - One health surveillance system
  - Selective use of NGS, using in-country sample referral
- Prioritize outbreak response in a One Health approach
  - Pen-side diagnostics?

    vs
  - Better sample referral

Finding the balance – DNO to improve routine surveillance

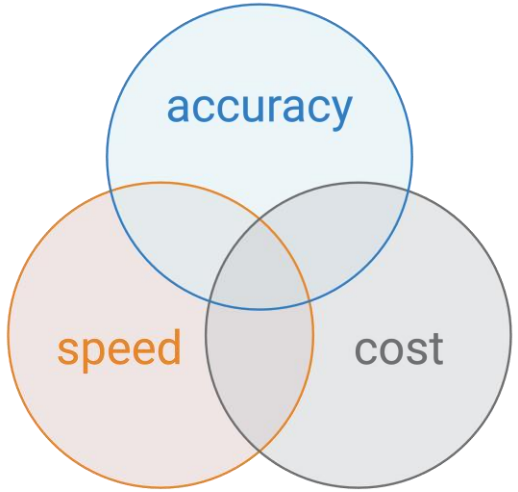
- Pen-side testing for knowns, further screening by PCR, etc.?
- Based on formal criteria for quality and cost effectiveness



Focused metagenomics data is used for detection, discovery, characterization and molecular epidemiology but ALSO refinement of the essential diagnostic list, continually reducing the need for the more expensive method and reducing TAT

Sustainability – identifying a fit-for-purpose genomics method

- One harmonized molecular method
  - Cost effective
  - User friendly
  - Sufficient to generate the necessary (high quality) data



But what is accuracy in sequencing??

- mainly depth and breadth of coverage

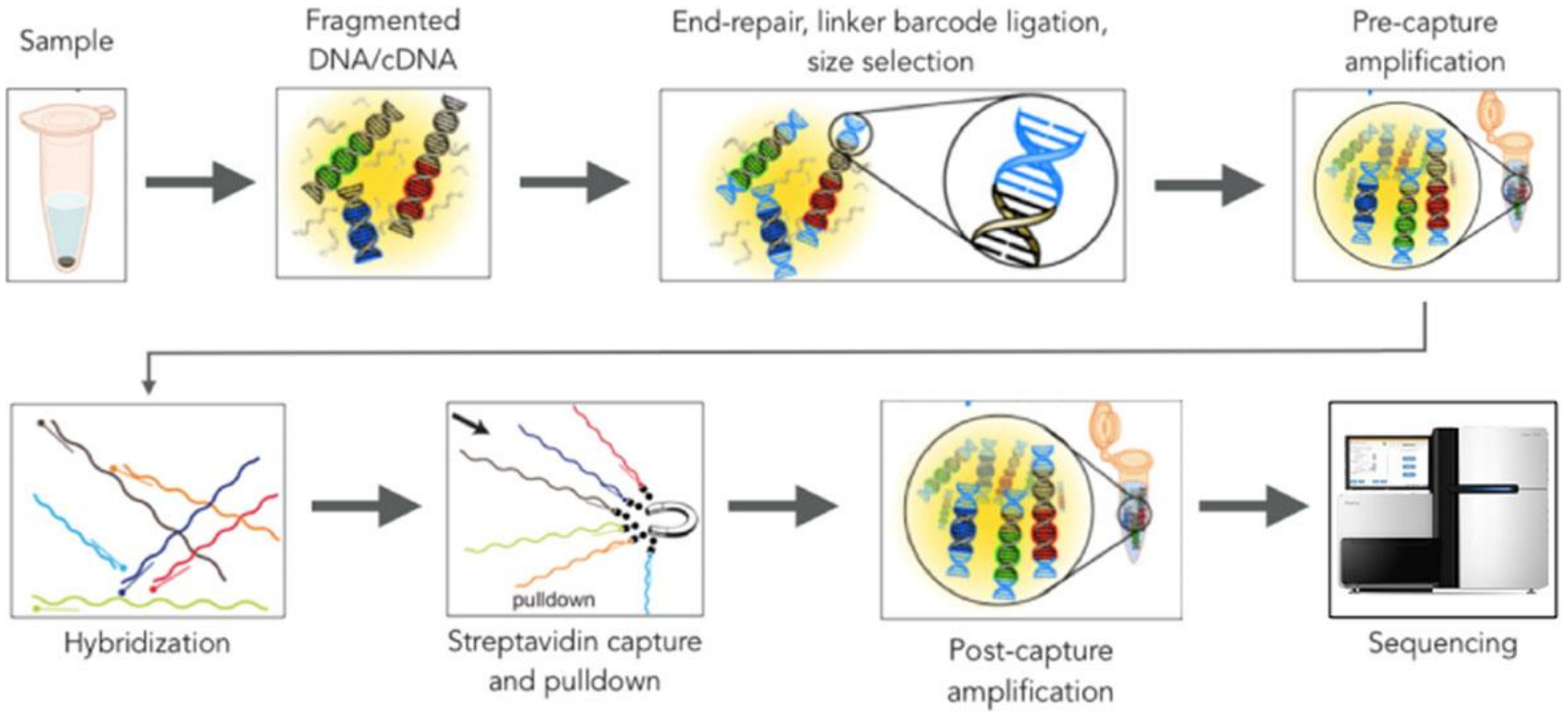
So many tools give great information but with every increase in granularity or completeness of information comes both greater cost and longer TAT

Where is the sweet spot??

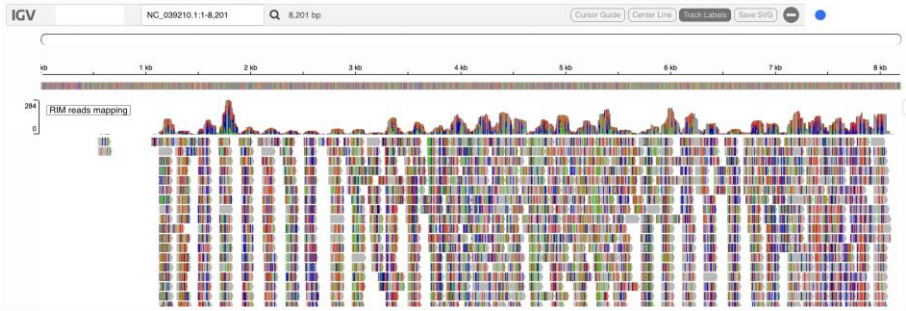
Cost effective doesn't mean cheap – it means fit for purpose

Virome Capture Sequencing – Vertebrates

- Capable of detecting and providing full sequence of all vertebrate viruses on GenBank and any up to about 40% divergent: pan-viral detection and discovery
- Greater sensitivity in some cases than PCR
- Efficiency, simplicity, cost effectiveness



Capture sequencing



Probes Mapping to FMD reference

- NOT cost effective for low sample volume (e.g., suspected intentional event)
  - Nonetheless sustainable if the system is routinely used for other things
- For forensic genomics, quality is paramount
- Capture sequencing is focused metagenomics –
  - More samples per flow cell -> more cost effective
  - Cleaner data -> simpler bioinformatics
  - Sample and platform agnostic – shared workforce and data management can support public health, One Health, forensics, etc.
  - Fit for purpose because even manipulated pathogens still have to function in the constraints of nature

Genomic “signatures”

- In the end the evidence that matters is genomic
- But expert genomic forensics services in every country may not be feasible
- Routine genomic diagnostics for public health and One Health is feasible
- Do DNO to ensure sustainability of routine use of genomics
- Get cost effective focused metagenomics in place in surveillance in production animal medicine, in clinical human medicine (and maybe clinical vet med??) and you have sustainable technical capacity in country to provide for forensic genomics

Virome

- We need more virome data but we need to get it cost-effectively
- Routine use for surveillance with the right assay will provide virome
- Virome matters both to continue to validate AI/ML models to detection manipulation AND to simply understand what is normal – when carrying out bioinformatics, what is effectively background "noise?"

Patrice Bonny, Julien Schaeffer, Alban Besnard, Marion Desdouits, Jean Justin Essia Ngang and Françoise S. Le Guyader. Human and Animal RNA Virus Diversity Detected by Metagenomics in Cameroonian Clams. Frontiers in Microbiology. 30 November 2021. doi: 10.3389/fmicb.2021.770385

Quality control

- The “kitome” or “lab-ome”
- Why sequencing for attribution or forensics must happen as close to the cases as possible – packaging, shipping and every sample manipulation incurs risk of contamination
- WOAH reference labs should develop methods and assist in implementation in member state lab systems – though NOT necessarily member state central veterinary labs

Key messages

- Consolidate genomics capacity within the member state in a One Health fashion
- Optimize the veterinary diagnostic network
- Select a cost-effective genomics method
- Ensure access to genomics for genomic forensics within the member state

- Conclusion: Deliberate adoption of NGS can achieve the One Health vision and can enable biosecurity in all its manifestations.

GAPP literature

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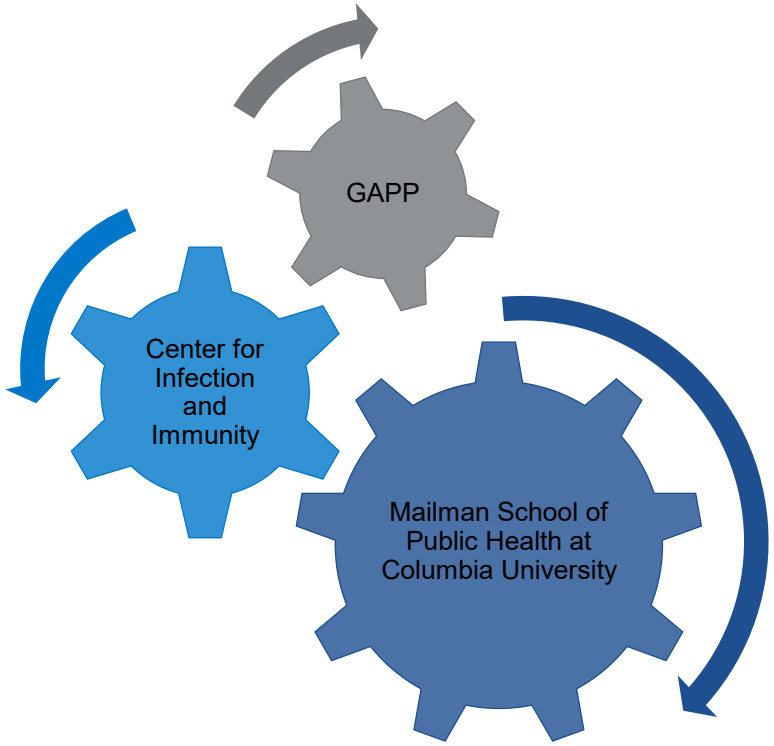

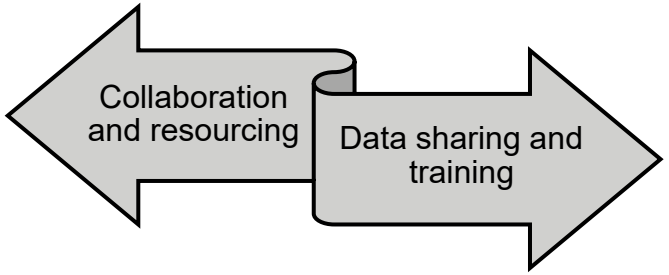

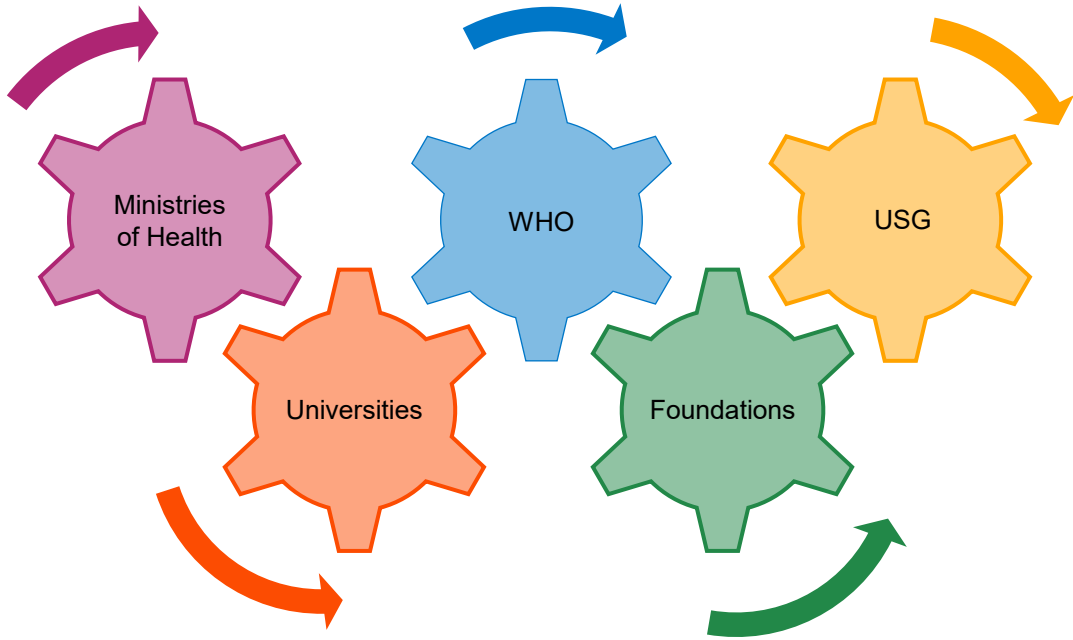

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