Sample collection, transportation and processing

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Why should we harmonize sample collection, transportation and processing?

- Allow easy aggregating/pooling of data from different trials to increase statistical power/easy systematic review process.
- Easily share experiences, work and reagents.
- Establish and maintain high quality processes leading to high quality samples.
- Make it easy for audits/evaluations.

How can we standardize the processes?

- Standardize SOPs for all procedures including phlebotomy, sample mixing, transportation temperature, duration of samples outside the body before storage, laboratory processes, sample storage, sample retrieval etc. Heparin/EDTA/ACD etc

- Standardize equipment for all/many laboratory tests and assays.
  - Does not mean that the equipment must be identical.

- Standardize common tests and assays.-Share reagents?

Standardization of processes- Experience with IDEA consortium, TOKOMEZAPlus and others

- Participating laboratories to send representatives to one member laboratory and agree on harmonized/standard SOPs for laboratory processes.
- Participants move back to their laboratories and implement the harmonized SOP and agree ranges.
- **Centralized laboratories** to set up EQA for the laboratories/subscribe to common international EQA such as EQAPOL.
- **Centralized laboratories** evaluate the results from the participating labs and checks the agreed ranges-Purpose is to work with the laboratory to investigate and improve the results.

PBMC- UVRI experience- out of outbreak

- ACD vacutainers- Venous blood-appropriate PPE
- Triple packaging.
- Evaluate sample quality-hemolysis?
- Ficoll-Paque (density 1.077g/ml)-Sugar density is affected by changes in temperature. Chelating agent Vs no chelating agent.
- Direct layering of blood on Ficoll-Paque.
- Centrifuge at 800g for 30 minutes.
- Harvest white PBMC band and **plasma.**
- Wash PBMC twice with HBSS and count cells using hemocytometer.

PBMC

- PBMC mixed in Serum 90% and DMSO 10%. (10 million cells per ml).
- Strata cooler/Mr. Frosty -80℃ for 24 hours and move to LN_2.-Gas phase?
- Split storage of samples.
- Evaluate quality indicators of PBMC isolation and cryopreservation.
- Number of recovered cells/ml of blood, colour of PBMC pellet, duration between freezing time and sample draw time etc.

Serum out of outbreak

- Collect using SST tubes-appropriate PPE, triple packaging.
- Centrifuge and store.

Serum during outbreak

- Collect using SST tubes-appropriate PPE, triple packaging
- Centrifuge in glovebox and store.

PBMC isolation and cryopreservation- TOKOMEZAplus SUDV trial during outbreak-Plans

- Collect blood samples from consented adults and children (6 years and above), 30 minutes after receiving the vaccine or no vaccine.
- Triple packaging of sample and transport the blood samples by road to UVRI- Appropriate PPE.
- Keep blood samples at 4℃ and do diagnostic PCR.
- If sample is non reactive- process samples using common protocol use appropriate PPE.-**First centrifugation outside the glovebox subsequent centrifugation in the glovebox until samples are ready to be moved out of the glovebox.**
- If sample is reactive- **destroy** the blood sample.

National biorepository for specimen

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Problem

- There are few standardized tests and reagents for the diagnosis of most of the VHF including MVD (antigen and antibody based).
- No standardized assays for the evaluation of immunogenicity of experimental VHF including MVD vaccines.
- No licensed therapeutics targeting VHF including MVD.
- Uncoordinated, poorly documented studies involving survivors and patients of MVD outbreaks.
- High demand for samples from VHF including MVD survivors.

Rationale

- To support national and global efforts towards research and development of new diagnostics, therapeutics and vaccines against VHF including MVD.

- We propose to set up a national repository for biospecimen from VHF patients and survivors including MVD.

Approach 1 (Patients)

- Integrate into the goals and objectives of all patient guidelines (MoH).

- Collect blood samples daily (20ml) from patients admitted in the treatment units.
- Plasma (EDTA)
- Serum (SST)
- DNA aliquots (DNA PAXgene tubes)
- RNA aliquots (RNA PAXgene tubes)
- Breastmilk
- PBMC

Approach 2 (Survivors)

- Integrate into the goals and objectives of all survivor guidelines (MoH).
- Work with existing survivors to collect blood volumes of up to 150mls from consenting survivors.
- 3 monthly for 1 year, every 6 months for 1 year, once a year for 3 years.
- 110mls- PBMC and ACD plasma.
- 25mls-Serum (SST)
- 7.5 ml DNA aliquots (3 DNA PAXgene tubes)
- 7.5 mls to process RNA aliquots (3 RNA PAXgene tubes)
- 120ml- Breastmilk

Storage, access and release

- Establish a national biorepository at CPHL and other sites including H3Africa Biorepository initiative (SBS, CHS, MUK) and UVRI
- Maintain an online catalogue of samples.
- Access will be through requisition to the DG via research pillar chair MoH
- Approved protocols and evidence of beneficence and non-maleficence.
- Demonstrate the beneficence and benevolence to the people of Uganda.
- Follow national guidelines for data and biospecimen access

Progress

- UNCST approached for joint review of protocol.
- UNCST requested biobanks to apply for certification first.
- CPHL/MoH is willing to offer mobile P3/P2 laboratory.
- Seeking for funding to operationalize the sample collection protocol for disease outbreaks.

Biosafety recommendations

- Laboratory workers involved in efficacy vaccine trials or providing support to therapeutic trials request to be vaccinated.
- PCR+ samples during efficacy trials need to be discarded therefore, downstream assays for immunological analysis cannot be performed.
- Biosafety inactivation and transportation protocols need to be considered first when setting up new supporting laboratories

Diagnostics recommendations

- There are two options of diagnostic assays to support the core protocols: Close systems (such as GeneXpert) and open systems (such as real-time PCR).
- Open systems are more flexible and more specific, but require intensive staff training, depending on the number of samples.

- Closed systems like GeneXpert may not be cost-effective. Depends on the scale of the trial, maybe ok for phase I, II not for phase III.

- In tropical settings GeneXpert may not be stable enough compared to open systems. –Power/electricity, disposal of cartridges etc.

Diagnostics recommendations

- Contamination issues (open vs close systems). Separate rooms for open system.
- Decisions open vs close depend greatly on experience of staff and existing facilities. Closed needs less experienced staff etc.
- Sample storage, biobank-capacity needs to be arranged in advance. Mobile lab for region affected by VHFs.

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