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°C for 24 hours and move to LN₂. -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- TOKOMEZApplus 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°C 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 VHF's.
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