Sentinel Surveillance of Acute Undifferentiated Febrile Illness in hospitalized patients

Royal Centre for Disease Control

Department of Public Health, Ministry of Health, Thimphu

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List of Abbreviations

AUFI	Acute undifferentiated febrile illness
CHIK	Chikungunya
DV	Dengue Virus
DNA	Deoxyribose Nucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
IPD	in-patient department
IgG	Immunoglobulin G
IgM	Immunoglobulin M
JE	Japanese encephalitis
PCR	Polymerase Chain Reaction
PI	Principle Investigator
REBH	Research Ethical Board Health
RT-PCR	Reverse Transcriptase – PCR
RCDC	Royal Center for Disease Control

Protocol Summary

Title: Sentinel Surveillance of Acute Undifferentiated Febrile Illness (AUFI) in hospitalized patients

Design: Prospective and observational study.

Population/Study Size: All cases that fulfills inclusion criteria

Objective: To ascertain common etiologic agents among acute undifferentiated febrile illnesses patients presenting to hospitals and require hospitalization.

Schematic of Study Design:

- Prospective enrollment of acute undifferentiated febrile illness identified among admitted patients after routine investigation at selected hospitals.
- Demographic, clinical information and samples including results of routine blood examination conducted for the patient during case investigation and management will be collected from enrolled cases.
- Laboratory assays for scrub typhus, leptospirosis, dengue, Japanese encephalitis, chikungunya and brucellosis pathogens will be performed at RCDC
- Analysis of data to determine incidence of pathogens causing AUFI's including clinical profile and routine blood examination results.

1 BACKGROUND

The acute undifferentiated febrile illnesses (AUFIs) are a common clinical problem in south Asia. [1] AUFIs represent a considerable burden of disease with diagnostic and therapeutic challenges. Empirical broad-spectrum antimicrobials are generally prescribed but with little evidence-based guidance on likely etiologies or potential treatment responses. [2] A variety of etiologies have been reported in patients presenting with acute undifferentiated fever in tropical areas. [1],[2],[3] Malaria, dengue fever, scrub typhus, other rickettsioses, leptospirosis, and enteric fever are common causes of acute undifferentiated fever, causing considerable morbidity, mortality, and economic burden. However, the etiologic spectrum of acute undifferentiated fever has been poorly characterized in developing and under developing nation in tropical areas because of limited diagnostic capacity. [4]

In Bhutan, the infectious causes of febrile illness remain poorly characterized, largely due to limited diagnostic and microbiological facilities. Febrile illness is one of the most common reasons for seeking medical attention, but there is limited information on the frequency of specific infections. Therefore, treatment of febrile illness/undifferentiated febrile illness is empirical and generally broad-spectrum antimicrobials are prescribed. Few sentinel surveillance conducted have documented the presence of infection with *Orientia tsutsugamushi*, the causative agent of scrub typhus and *Rickettsia typhi*, the causative agent of murine typhus. Scrub typhus has been confirmed as febrile illness outbreaks in community and schools. Although only few leptospirosis cases are diagnosed in human but leptospirosis prevalence is very high in domestic animals across the country. Arboviruses (Japanese encephalitis (JE), Dengue (DV), Chikungunya (CHIK) causing febrile illnesses are also reported in the country.

The provision of adequate epidemiological data for common pathogens will enable resources to be directed towards key areas and will be of practical importance to clinicians. For populations where microbiological facilities cannot be permanently established, validated clinical predictors may help guide therapeutic interventions.

1.1 Rickettsia

Rickettsiae are obligate intracellular parasites. The majority of rickettsiae are maintained in nature by a cycle involving an arthropod vector and animal reservoir. Infection is usually conveyed to humans through the skin from excreta or saliva of arthropod vectors.

In humans, rickettsiae multiply in vascular endothelial cells especially in capillaries, resulting in a systemic illness with pathologic lesions of the skin, central nervous system, heart, lung, kidney, and skeletal muscles. Common clinical findings include fever, severe prostration, mental disturbance and often rash. An eschar

may be seen in tick and mite born typhus. Species specific IgM and IgG antibodies may be detected by ELISA techniques and PCR.

1.2 Leptospirosis

Human infections occur primarily through contact with contaminated water or through contact with infected animal urine or tissues. *Leptospira* enter through abraded skin or mucous membranes. Certain occupational groups are at especially high risk especially veterinarians, and agricultural and sewage workers. Common clinical features include fever with sudden onset, headache, chills, and severe myalgias. Physical examination findings may include relative bradycardia and conjuctival suffusion. Less common findings include pharyngeal injection, cutaneous hemorrhage, and skin rash. Weil's syndrome occurs in 1-6% of cases and is a severe form of leptospirosis that manifests with jaundice, azotemia, hemorrhage, anemia, altered consciousness, and fever.

Diagnosis is made by culture or serology. Antibodies to the organism appear between day 6 and day 12 of illness, and the antibody titer can rise by four fold over the course of the illness.

1.3 Arboviruses (Japanese encephalitis (JE), Dengue (DV), Chikungunya (CHIK))

JE is an acute inflammatory viral disease of short duration involving the brain, spinal cord, and meninges. Severe infections are usually marked by acute onset, headache, high fever, meningeal signs, stupor, disorientation, coma, tremors, occasional convulsions, and spastic paralysis. Mortality rates can be as high as 50%, with JE.

Dengue and Chikungunya are acute febrile diseases, characterized by sudden onset of fever, headache and often retro-orbital pain, joint and muscle pain and rash. Both viruses are transmitted by *Aedes* mosquitoes and can cause severe illness and death. Although dengue virus is commonly transmitted by *Ae. aegypti, Ae. albopictus* is also a competent vector.

Arboviruses are diagnosed by identifying a clinical presentation with the detection of specific IgM antibody by ELISA and PCR.

1.4 Brucellosis

Brucellosis is a highly contagious zoonosis caused by ingestion of unpasteurized milk or undercooked meat from infected animals, or close contact with their secretions. It is also known as undulant fever, Malta fever, and Mediterranean fever. [(5)]

B. melitensis is the most virulent and invasive species; it usually infects goats and occasionally sheep. The symptoms are like those associated with many other febrile diseases, but with more muscular pain and

night sweats. Brucellosis induces inconstant fevers, miscarriage, sweating, weakness, anemia, headaches, depression, and muscular and bodily pain.

Definite diagnosis of brucellosis requires the isolation of the organism from the blood, body fluids, or tissues, but serological methods may be the only tests available in many settings. Identification of specific antibodies against bacterial lipopolysaccharide and other antigens can be detected by the standard agglutination test (SAT) and ELISA. SAT is the most commonly used serology in endemic areas. An agglutination titer greater than 1:160 is considered significant in nonendemic areas and greater than 1:320 in endemic areas.

2 SCIENTIFIC RATIONALE

Acute undifferentiated febrile illness is one of the most common reasons for seeking medical care in Bhutan. Individuals presenting to health facilities with AUFI are treated empirically with the underlying illness remaining undiagnosed. Therefore, it is unclear what fraction of febrile illnesses are due to bacterial, viral, rickettsial, spirochetal, or parasitic pathogens. A general unavailability of diagnostic tests accounts for this occurrence. The recent development of rapid antigen and specific IgM detection assays has simplified, and increased access to, diagnostic algorithms.

RCDC has conducted the sentinel surveillance in 2016-2017 and found rickettsiae, leptospira, and arboviruses are common cause of AUFI. However, to understand the seasonality, disease burden caused by above pathogens, systematic surveillance has to be carried out. The year-round epidemiologic data collected from AUFI admitted patients in the hospital for different pathogens and diseases caused will assist clinicians in their diagnoses and subsequent therapeutic interventions even when laboratory resources are lacking.

2.1 Potential Risks and Benefits

2.1.1 Potential Risks

This surveillance involves only collecting clinical information and blood tests result already performed as per of routine investigation is thereby considered as minimal risk to the subjects. Specimen preparation from collected blood samples will be performed by trained medical laboratory technicians. Every effort will be made to maintain subject confidentiality.

2.1.2 Potential Benefits

There may be no immediate benefits to all participants in this surveillance. In some cases, subjects may benefit by determining accurate diagnosis early enough for appropriate intervention and potentially

avoiding complication and life-threatening situation.

3 OBJECTIVES

3.1 Primary Objective

- To determine the etiology of acute undifferentiated febrile illnesses using appropriate diagnostic techniques to diagnose rickettsial, leptospirosis, typhoid/paratyphoid, brucellosis and systematic diseases caused by arboviruses.

3.2 Secondary Objectives

- To characterize the clinical profile of AUFI caused by different pathogens
- To study blood profile (hematology and biochemistry) of AUFI
- To study treatment approach used by clinicians for AUFI

4 METHOD

4.1 Design

This protocol describes prospective surveillance of **acute undifferentiated febrile illness (AUFI)** among hospitalized patients in selected hospitals for study of common pathogens causing AUFI. The overall surveillance design will be followed as per the Appendix 1.

4.2 Case definition of AUFI

Defined as fevers that typically do not extend beyond a fortnight, and lack localizable or organ-specific clinical features on history, physical examination and routine investigations.

4.2.1 Inclusion Criteria

- Male or female greater than or equal to 6 months of age;
- Possessing a fever (axillary temp. ≥37.5°C) on presentation at hospital
- Duration of fever less than 14 days
- Hospitalized
- No detection of specific single organ involvement by history, physical examination and routine investigations (CBC, RFT, LFT, Widal and urine analysis)
- Multi system involvement

4.2.2 Exclusion Criteria

- Less than 6 months of age;
- Localized cause of fever (fever with symptoms, signs or an investigation that localized the source of infection to skin, soft tissue, respiratory, gastroenteritis, cellulitis, urinary tract infection, septic arthritis, and peritonitis.
- Patients detected to have a malignancy or autoimmune disorder

4.3 STUDY SETTING

4.3.1 Sampling Strategy and Sample Size

Surveillance plan to enroll AUFI cases among hospitalized patients based on the inclusion criteria and not meeting exclusion criteria from identified 11 sentinel hospitals namely; **JDWNRH, GRRH, MRRH, Phuentsholing, Paro, Punakha, Samtse, Tsirang, Trongsa, Trashigang and SamdrupJongkhar hospitals** (Figure 1). The sentinel site selection is based on past history of high acute febrile cases reported by hospitals including referral hospitals. Sample size is not relevant for this surveillance as this is a descriptive study in the absence of previous clinical and laboratory data.

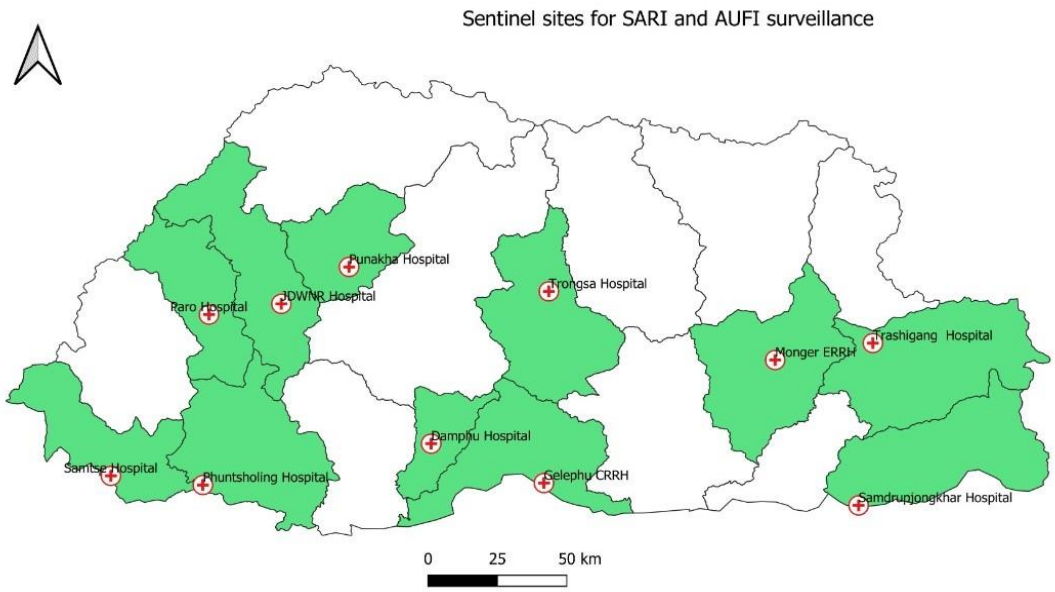

Figure 1: Sentinel surveillance site for AUFI and SARI

4.4 Subject and Specimen Identification

The AUFI case number will be provided, the case number or barcode number shall consist of letters to

designate the type of disease surveillance, surveillance site and followed by the running subject number (example **AUFI-PUN-001, 1st case of AUFI from Punakha hospital)**. The case ID number will be barcodes and will identify the patient on demographic and clinical data form, laboratory collection data form and specimen coming from at each site. Results by case number will be sent to the enrollment site to notify the physician of the patient's test results. The patient's physician may notify the patient of the test results if required and requested by the patient. Only persons collecting patient's information, laboratory results, samples at each site, the PI and the database manager will be authorized to access the data. Subject names will not be analyzed in the study. The data collected will be destroyed three years after completion of surveillance. At the end of the surveillance, specimens will be further stored for 10 years and then destroyed.

4.5 Case enrollment and Data Collection

AUFI cases will be enrolled primarily from the in-patient department (IPD) after routine examination and applying inclusion and exclusion criteria. The patients will receive medications and treatment as prescribed by the clinician in the hospital based on investigation. Clinicians and laboratory staff at respective hospitals will be briefed on the purpose, objectives, and methods of the surveillance including storage of blood samples collected for investigation until case is identified as AUFI for enrollment by healthcare professional.

After AUFI case is identified by the clinicians or nurses, the concerned clinician or nurse will fill up the demographic and clinical data form (Appendix 2) and inform laboratory personnel about the case. The laboratory personnel or nurse will then collect blood samples adequate for both routine investigation and surveillance and store serum and RBC at 2-8°C for shipment to RCDC.

4.6 Informed Consent

The surveillance is among hospitalized patients and the cases will be enrolled only after routine investigation and the clinicians identify a patient as AUFI case. Data and sample collection for surveillance will be done from history taken during admission, admission chart and sample collected for routine investigation. Since, the enrolled cases will not be subjected to additional information collection and invasive procedures for the purpose of this surveillance, informed consent may not be necessary.

4.7 Specimen Collection

No separate sample collection will be done for the surveillance purpose from the enrolled cases. RCDC will train lab staff from each sentinel hospitals to prepare blood DBS from EDTA blood sample or plain tube and aliquot of serum from clotted blood sample of the identified AUFI cases. All sample collection should be preferably carried out prior to the institution of antibiotics to the patients. Identified hospital

laboratories should retain the blood samples for 48-72 hours in case additional investigation may be required.

For the preparation of the DBS, the EDTA blood should not be stored for more than 24 hours. Request for fresh samples if the saved blood has exceeded 20 hours of storage or the collected blood is insufficient for AUFI test.

4.8 Storage and Transportation

DBS and serum samples will be stored at 2-8^{o}C refrigerator and shipped to RCDC weekly as per the existing shipment mechanism for other surveillance conducted by RCDC in appropriate cold chain. The samples will be stored at -70^{o}C freezer at RCDC.

4.9 Diagnostics

4.9.1 Serologic tests

4.9.1.1 Rickettsial

ELISA tests will be used to detect specific IgM antibodies against rickettsia; scrub typhus, murine typhus and spotted fever. The test will be performed as per the manufactures insert and SOP prepared at RCDC lab.

4.9.1.2 Leptospirosis

ELISA tests will be used to detect specific IgM antibody against. The test will be performed as per the manufactures insert and SOP prepared at RCDC lab. Confirmation and serovars will be done using MAT

4.9.1.3 Dengue, Chikungunya and Japanese Encephalitis,

ELISA test will be used to test for specific IgM antibodies against dengue, chikungunya, and Japanese encephalitis.

4.9.1.4 Brucellosis

IgM antibody against brucella will be tested using a suitable serological assay, following the instructions provided by the manufacturer in the kit insert.

4.9.2 PCR tests at RCDC

We will use molecular approaches to detect rickettsial agents and identify the pathogens. DNA will be extracted from 300 µl EDTA blood samples using QIAamp Mini blood kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol. *Rickettsia-Orientia* duplex nested PCR targeting genus specific

genes of rickettsiae and *Orientiatsutsugamushi*, 17-kDa and 56 kDa, will be performed as previously described.[29] DNA sequencing of the amplified product will be used to specifically identify species of rickettsiae. Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA) enzyme mixture will be used in the PCR reaction. Additional rickettsial gene fragments, 630 nt-*ompA* (nt 70–701) and 945 nt-*gltA* (RpCS.193F-5´-GTAGGGTATCTGCGGAAGCC-3´, RpCS.1143R-5´-GAGCGAGAGCTTCAAGTTCTATTGC-3´), will also be amplified.[29] All amplicons will be excised from agarose gels, purified by QIAEX II Gel Extraction Kit (QIAGEN), and then sequenced. Obtained nucleotide sequences will be analyzed by Sequencher (Applied Biosystems, Foster City, CA) and BLAST software program from the GenBank database. Dengue PCR will be performed as per the existing PCR SOP at RCDC

4.10 Specimen Disposition

A specimen repository will be maintained at RCDC for a maximum of 10 years and then properly destroyed through autoclaving or other appropriate measures. Use of specimens for other study not covered in the surveillance protocol will only be conducted in separate protocol approved by the Ethical Review Committee.

4.11 Training

RCDC will train relevant hospital staff (clinicians, nurse and laboratory technicians) in the surveillance sites on patient enrollment, specimen collection, storage, transportation of specimens, and universal precautions before the surveillance starts.

4.12 Equipment & Supplies

Specimen transmittal consumable, forms and shipment boxes will be supplied by RCDC. The specimens will be batched and shipped weekly to RCDC in ice pack.

4.13 Data Handling

*4.13.1 **Disposition of Data***

Source data sheets (demographic and clinical form) and diagnostic test results will be maintained in locked file cabinets at RCDC. The original copies of the clinical form will be entered into a database and kept at RCDC. All documents (clinical forms and test results) will be destroyed within one year after completion of the study. All specific information pertaining to patients will remain confidential.

Data will be compiled, computerized, carefully edited using standard procedures and analyzed RCDC. Database files will be kept on a password-protected computer at RCDC in a locked room and with a limited

access for authorized personnel only. Database files will contain study number, age, and sex but not name or other identifiable data. Data used for analyses or publication will be based on study subject number and not contain individual identifying information.

4.13.2 Data Analysis

Data analysis will consist of descriptive statistics and standard statistical tests. Categorical data will be analyzed by the use of either Chi-Square (expected cell frequency > 5) or Fisher's Exact Test (expected cell frequency <=5). All samples including those with cold chain breaks will be included in the analysis.

4.13.3 Reports

Both interim and final reports will be made available to all investigators listed on this protocol.

5 REFERENCES

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6 APPENDIX

6.1 *Appendix 1*. **Study Flowchart**

At hospital (Inpatient ward) – Clinicians/Nurses will

Identify AUFI cases

Collect demographic and clinical information in AUFI form

At hospital lab – Laboratory personnel or nurse

Collect adequate blood sample volume for routine investigation and surveillance.

Centrifuge and aliquot serum in micro-vial tube and keep RBC in the same tube

Laboratory personnel

Ship serum and RBC in proper cold chain to RCDC

6.2 *Appendix 2.* **AUFI Form**

AUFI form (Acute undifferentiated febrile illness)
(Version 1)

Case No. (Barcode): [ ]

1. PATIENT INFORMATION

Name of Health Centre:		
Patient Name:	Age:	Gender:
CID:	Contact Number:	
Occupation:	Residential Address:	

2. CLINICAL INFORMATION

Fever: ☐ Yes ☐ No If Yes, Temperature: .....

Date of fever onset (dd/mmm/yy): _____ Duration of fever: .....

Symptoms	Yes	No	Symptoms	Yes	No
Chills			Chest Pain		
Malaise			Nausea		
Muscle Aches			Vomiting		
Headache			Abdominal Cramps		
Retro-orbital Pain			Diarrhea		
Joint Pain			Loss of consciousness		
Fatigue			Seizures		
Anorexia			Confusion		
Conjunctivitis			Stiff Neck		
Coryza			Jaundice		
Rhinorrhea			Rigors		
Sore Throat			Sweating		
Cough			Rash		
Wheezing			Easy bruising		
Shortness of Breath			Bleeding		

Others: specify.....

3. CO-MORBIDITIES

☐ None ☐ Diabetes ☐ Heart Disease ☐ Hypertension ☐ Seizure Disorders
☐ Asthma ☐ Kidney Disease ☐ Liver Disease

Patient Outcome: ☐ Referred ☐ Recovered ☐ Died

4. Travel History in last 14 days? ☐Yes ☐No
If Yes, country or place visited: .....Travel Date: .....

Any contact with person having similar symptoms in the past 14 days? ☐Yes ☐No
If Yes, date of contact (DD/MM/YY): .....

5. Laboratory Blood Specimen Collected

Sample ID: [ ] Collection Date: .....

Type of Specimen
☐ Whole blood (red cap tube)
Note: Whole blood will be centrifuged by Lab and send both Serum (>1 mL) and RBC to RCDC
* Please attach CBC or any lab test reports performed at the hospital along with this form and specimen.

Advised by: _____
Sample Collected by: **Contact No.:** .....

6.3 *Appendix 3.* **Sample and Cold Chain log Form**

Sl. No.	Specimen No.	Collection Date	Shipment date	Forms included (put "√ " mark)		Shipped by
				Clinical data	Laboratory data	

*specimens can be kept in a refrigerator (between 2-8 ^{o}C) for not more than 72 hours.

Demographic /clinical form should accompany this table.

At study site: Shipment prepared by: ……..Date :……

At RCDC: Shipment received by: ……...

Date …….(dd/mmm/yy) Time :……..(if needed)

Condition of the samples when received:_____

6.4 Appendix 4. DBS preparation Standard Operating Procedure (SOP)

1. Purpose and scope:

This SOP describes the venous blood sample collection from patients and or subject for the study.

2. Safety Precautions

- Once survey participant has been selected, choose a comfortable place to set up the biological sampling station.
- Lay out a clean disposable mat with all the equipment necessary to collect blood samples.
- For each participant, wear new gloves and conduct all procedures on a clean disposable mat.
- After transferring any biological sample into the whatman paper, immediately label all samples accurately.
- Assume that all human blood is potentially infectious for HIV, hepatitis, and other infectious agents.
- Practice Universal Precautions using gloves, eye protection, and lab coats.
- Always use sterile, single-use, disposable supplies for sample collection.

3. Introduction:

Venous blood is suitable sample for analysis of any lab parameter for diagnosis and study purpose. Blood can be either collected in test tube containing; citrate, heparin, or EDTA or without anticoagulant based on requirement. The purpose for venous blood collection for this study is to extract the total DNA (e.g., genomic, viral, mitochondrial) and purified from whole blood.

4. Responsibilities:

4.1. Any health workers or professionals responsible for collecting venous blood sample for this study in the community MUST comply with the SOP.

5. Materials and Equipment

Materials

. 5mlsterile disposable syringe and needle;
. Tourniquet
. Sterilizing swabs
. Specimen labels
. Band-aid
. Zip-lock plastic bags
. Sample line Form
. Whatman filter paper
. Disposable gloves
. Sharp container
. Desiccant tablets

Equipment

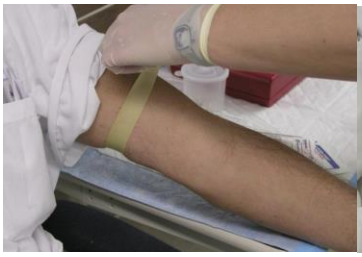
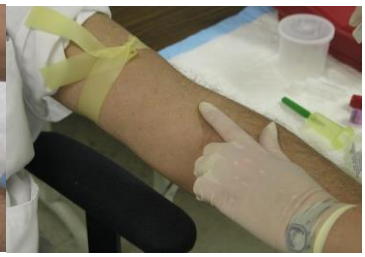
Not required for this sample collection for this study

6. **Procedure**

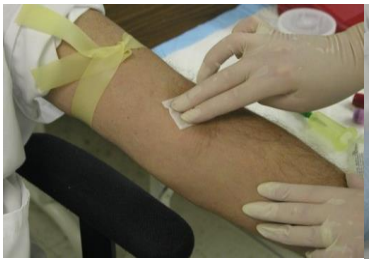
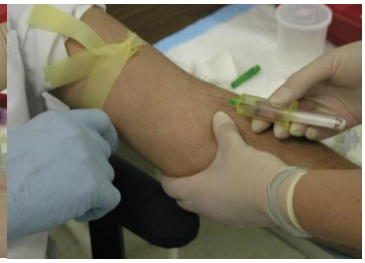
6.1 Venous blood collection

- Make sure participant is sited comfortably.
- Lay out all blood collection supplies and necessary labels. Assemble needle and syringe.
- Examine both arms to find the best vein.
- Locate the puncture site; apply the tourniquet.
- Wipe the area in a circular motion making sure the area is thoroughly cleaned and allow it to dry.
- If it is necessary to feel the vein again, do so, but cleanse the area again with an alcohol wipe and dry with gauze.
- Fix the vein by pressing down on the vein about 1 inch below the proposed point of entry and pull the skin taut.
- Remove the needle shield.
- Approach the vein in the same direction the vein is running, holding the needle so that it is at an approximately 15°angle with the subjects arm.
- Push the needle, with bevel facing up, firmly and deliberately into the vein.
- Withdraw 2-3ml blood. If the needle is in the vein, blood will flow freely into the tube.
- After collection is completed, loosen the tourniquet.
- Withdraw the needle. When the needle is out of the arm, press gauze firmly on the puncture.
- Use a cotton swab to apply pressure to the venepuncture site until bleeding stops and apply a band-aid.
- Dispose the needle in the sharp container

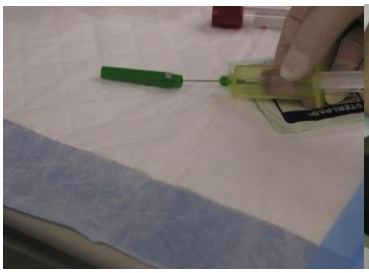
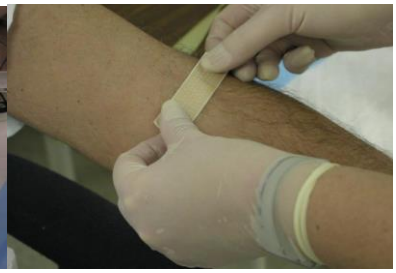
Step1 **Step 2**

Step 3 **Step 4**

Step 5 **Step 6**

Step 7

6.2 Dried blood spot preparation

- Label the whatman filter paper provided with subject identification number.
- Place whatman filter paper on the clean area (top of the tables, books or any hard surface)
- Drop venous blood from syringe (100 ul) on whatman filter paper slowly
- Prepare at least 3 spot on the same whatman filter paper from each subject blood sample
- Allow the dry the blood spots in the room. To dry the blood spot completely, it may take more than one hours.
- After it is dried, put the whatman filter paper in the ziplock bag. Batch dried blood spot in 5 number

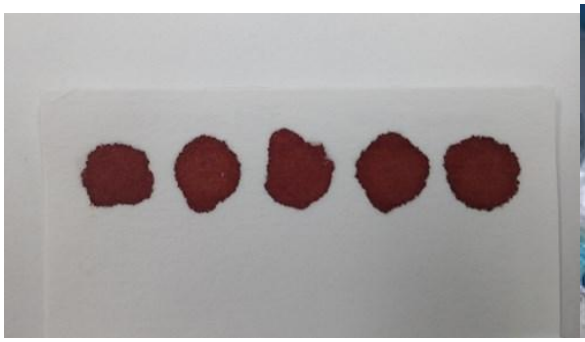
in each ziplock bag

- Place 4-5 piece of desiccant tables provided and seal the ziplock bag.
- Placed the ziplock bags in the envelop and ship it to the RCDC with sample line list form as per the study protocol.
- Label the ziplock bag with sample ID of DBS packed inside the ziplock.
- Pack the ziplock bags in the envelope for shipment .

Whatman filter paper grade 3

Preparation of DBS

Drying of DBS



Packaging of DBS