



**GOVERNMENT OF RWANDA
MINISTRY OF HEALTH
RWANDA BIOMEDICAL CENTER
NATIONAL REFERENCE LABORATORY**

BIOLOGICAL SAMPLE TRANSPORTATION WITHIN THE NATIONAL LABORATORY NETWORK

April, 2012

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FOREWORD

The National Reference Laboratory (NRL) was established in July 2003, enacted by law number 38/2007 and published into the “Official Gazette of the Republic of Rwanda” of 04 March 2008.

The vision of NRL is enshrined in the national medical laboratory policy which aims to achieve “*a healthy system with comprehensive laboratory services which are accessible and affordable to promote the well-being of the population*”. This vision is aligned with the national commitments in the Economic and Poverty Reduction Strategy (EDPRS) covering the period 2008-2012. This strategy is further aligned with the global Millennium Development Goals (MDGs) especially, MDG 6 on combating HIV/ AIDS, Tuberculosis, Malaria and other diseases.

The mission of NRL is “*to protect and promote health and quality of life in Rwanda by supporting the health services delivery in surveillance, prevention, diagnosis and management of diseases, through provision of quality and assured laboratory services at all levels of health care, in effective, efficient, and sustainable manner*”. The achievement of this mission will contribute to the attainment of the wider health sector aspirations for the country as enshrined in the national health policy and the Health Sector Strategic Plan (HSSP).

As stated in the NRL vision and mission, the service accessibility and the well-being of the population through provision of quality services are the most important and desirable goals to be achieved since they comply with population needs and demands. As an important part of the quality, the rapidity of services delivered to the population reflects the real effectiveness of underlying activities. As a laboratory, the NRL deals with patients samples from the whole Rwanda Lab network. As patients samples are laboratory substrates that must be well taken, packaged, transported and correctly tested to report correct results, the sample pathway has to be considered as one of the most important stapes. That is why the NRL set up a sample transportation project to reduce the overall turnaround times of results and consequently to decrease the delay of treatment.

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ACRONYMS AND ABBREVIATIONS

CHUB: Centre Hospitalier Universitaire de Butare
DH: District Hospital
EID: Early Infant Diagnosis
HC: Health Center
HIV: Human Immunodeficiency Virus
IHDPC: Institute of HIV/AIDS and Disease Prevention and Control
MDR TB: Multi Drug Resistant Tuberculosis
MOH: Ministry of Health
NRL: National Reference Laboratory
RBC: Rwanda Biomedical Center
SOPs: Standard Operating Procedures
EDPRS: Economic Development and Poverty Reduction Strategy
MDG: Millennium Development Goals

1. INTRODUCTION

In the interest of public health, human pathological specimens need to be transported safely, timely, efficiently and legally from the place where they are collected to the place where they will be analyzed. Regardless of the presumed infection status of the sample, any specimen of human origin should be packaged and transported in such a way that protects those engaged in transportation from the risk of infection. Risks of infection for personnel involved in transport must be avoided by all means. This proposal describes a plan, developed in collaboration between the Rwanda Ministry of Health and the National Reference Laboratory, to pilot a transportation network for transporting samples for Early Infant Diagnosis of HIV (EID), viral load (VL), sputum samples for culture, and others epidemics to the various District Hospitals and the NRL, for the purpose of performing essential laboratory tests in Rwanda. This specimen transportation network will expand on the already robust system of transporting medical samples from various health centers to District Hospitals throughout the country. The plan aims to expand access to laboratory tests that are essential in Rwanda for the diagnosis and monitoring of HIV, TB, malaria, and other epidemics diseases.

2. BACKGROUND

In Rwanda, there is currently a robust system of transporting medical specimens from various health centers to District Hospitals throughout the country. Many health centers transport specimens to a District Hospital by motorbike once a week, and receive results within 1-2 weeks. This system includes CD4 absolute count and percentage, clinical chemistry, and hematology for the monitoring of HIV-positive patients, as well as many other essential tests. However, for other more specialized laboratory tests that are only available at the NRL, such as Early Infant Diagnosis of HIV (EID), viral load (VL), and sputum for culture and Drug Susceptibility Testing (DST), transportation of samples is conducted in an ad hoc manner by the various health care facilities. Consequently, many patients do not have reliable access to these tests, or where there is access, turnaround times are often extremely long because health care facilities lack the resources to make frequent trips to the NRL. Many health centers only transport Dried Blood Spot (DBS) samples for EID once a month or less, when they travel to NRL for quality control procedures, many infants are lost to death or attrition without known HIV status. For viral load, health centers must transport samples within the 6 hour collection period of sample stability for plasma. Consequently, patients in most geographic areas outside of Kigali do not receive viral load testing at all because the health facilities are unable to collect and transport the blood within 6 hours. MDR TB samples also have short sample stability, and require testing at specialized labs.

To ensure that health centers and District Hospitals have reliable sample transportation system for a wider range of test types, a coordinated approach is necessary.

By partnering with the various health centers, District Hospitals, and a third party vehicle provider, RBC/ IHDPC /NRL division will be able to provide a reliable service of transporting

samples that will greatly improve patient access to timely diagnostics and quality of care. Additionally, healthcare workers will no longer be forced to spend much time transporting samples themselves, keeping them away from their site-level responsibilities. District Hospitals will provide the link between health centers and the NRL by providing a midpoint in the transportation of samples, making the various levels of healthcare facilities more interconnected. The RBC/ IHDPC /NRL division will manage this sample transportation pilot program centrally, and the program will benefit from greater expansion over the entire network of healthcare facilities in Rwanda.

3. PROPOSED PROJECT PLAN

3.1. SUMMARY

The National Reference Laboratory, with the financial and technical support from the MOH and partners, proposes to establish a sample transportation network system by first conducting a pilot phase study on a limited number of District Hospitals, and then expanding to all District Hospitals. Transportation services will be supplied by public chartered transport companies during the first period and by RBC/IHDPC/ NRL vehicles when they will be available. The vehicles will collect samples from various District Hospitals, and deliver them to the NRL twice a week for each itinerary. Drivers will be trained on regulations and procedures of sample transportation.

3.2. FREQUENCY OF TRANSPORTATION

The samples from health centers will continue to be sent to district hospitals by existing methods, and vehicles will pick them up from District Hospitals and deliver them to the NRL twice a week, depending on various factors, including patient volume, distance from the NRL, sample availability, tests requested, and budget on hand. Before the introduction of the system, the District Hospital authorities will be briefed and ideas exchanged in order to have a durable and sustainable system.

3.3. DISCUSSION OF TRADEOFFS OF VARIOUS TRANSPORTATION MODELS

There are many different models of sample transportation that were considered in the development of this proposal. Because many health centers currently send their samples to District Hospitals by motorbike, ambulance or other type of site vehicle, this model was considered for expansion to NRL. However, this was found not to be cost-effective because of the large number of vehicles that would be required. Public transportation was considered as another option, but was not able to provide a solution to the concerns regarding health and safety regulations of transporting blood samples.

Various courier services were also considered, but these also presented safety and liability concerns because they would transport samples with other cargo and passengers.

Finally, it was determined that the most feasible model was to rent dedicated vehicles from a supplier for a fixed cost per day, with the vehicles traveling from a central location in Kigali, making round trips to an average of three District Hospitals per trip. That rent is supposed to be done until the RBC/ IHDPC/ NRL will have its own vehicles.

Adding some tests like Early Infant Diagnosis for HIV, viral load, MDR TB and other epidemics to the scope of sample transportation creates new tradeoffs for the District Hospitals that act as a midpoint between the NRL and Health Centers. District Hospitals currently receive samples from Health Centers for CD4, chemistry, hematology, and other types of tests, to be tested on-site at the District Hospital. It is in their interest to spread this volume of samples throughout the week, so that the testing can be completed with minimal burden on test operators. For example, a District Hospital might currently receive samples from five different health centers, one on each day of the week. However, if health centers begin to send other types of samples, such as EID, viral load, and MDR TB, that need to be passed on from the District Hospital to NRL the same day they are received, the District Hospital will want to receive these samples as few times per week as possible. A greater number of trips per week from District Hospital to NRL will increase the time and cost of the sample transportation program.

To address these tradeoffs, each District Hospital will need to find the optimal balance between making more trips per week to NRL in order to spread its own testing activity throughout the week, and making fewer trips per week to keep costs down. This optimal balance will be determined by the volume of tests that must be conducted at the District Hospital each week, the number of health centers that refer to each District Hospital, and other factors.

Some District Hospitals can set up schedules so that its various health centers send CD4, chemistry, and hematology every week, but send EID, viral load, and MDR TB samples only every second week. This will allow District Hospitals to make only two trips to NRL every two weeks, although it still receives samples for on-site testing spread across two times per week. The downside is that each health center will only get access to transportation to NRL for the more specialized tests every two weeks. Other District Hospitals may decide that there are sufficient patients in the district to justify providing them sample transportation opportunities every week.

4. TECHNICAL SPECIFICATIONS

Today, thousands of samples of infectious substances need to be shipped and are shipped daily around the world. Human specimens are collected and shipped for a variety of reasons, including disease investigations, clinical trials, surveillance studies, anti-doping testing, routine analyses, etc. Regular and occasional shippers consign infectious substances for transport on a daily basis. These include the pharmaceutical industry, health care facilities, diagnostic and research laboratories, medical practitioners, and individual patients.

In the interest of global public health, human and animal specimens need to be transported safely, timely, efficiently and legally from the place where they are collected to the place where they will be analyzed. Regardless of the presumed infection status of the patient, specimens of human and animal origin should be packaged and transported in such a way that protects those engaged in transportation from the risk of infection. Risks of infection of personnel involved in transport may not be fully eliminated. However, they can undoubtedly be kept to a minimum level. In addition, damage to packaging also means that samples dispatched for urgent tasks like analyses are unlikely to arrive at their destination on time. In order to make appropriate decisions, shippers must understand their responsibility and obligation to be familiar with regulatory requirements. Dangerous goods regulations require all personnel involved in transport to undergo appropriate training. Appropriate training and education, commensurate with the shipper's responsibilities, will provide the shipper with the necessary degree of familiarity with applicable requirements, addressing identification, classification, packaging, marking, labeling and required documentation for the transport of infectious materials.

The international regulations for the transport of infectious materials by any mode of transport are based on the recommendations of the United Nations, particularly by the Committee of Experts on the Transport of Dangerous Goods. Standard precautions applying to blood, all body fluids, secretions, excretions (except sweat), non-intact skin, and mucous membranes will be applied in different Standard Operating Procedures (SOP's) in the Laboratory Network in Rwanda (HIV, TB and other samples).

As a consequence, health care providers will be encouraged to wear protective clothing, including gloves, gowns, and masks to protect themselves, the patients or materials, and the environment from infections. When shipped, the specimens will be in safe containers that will not be contaminated on the outside. Thus the shippers do not have to wear protective equipments.

For transportation by air/or international transport of strains or specimens, the National Reference Laboratory will plan to have a contract with an international air carrier which meets the packaging instruction (PI) 650.

4.1. TRANSPORTATION CONDITIONS

Currently, for in-country transport from a health facility to a laboratory or from one laboratory to another, transportation methods from the hospitals and laboratories or other approved agencies or organizations have been used (e.g. public transport companies). The principle of safe transport by this means is the same as for international transport; the materials should not have any possibility of leaking from the package under normal conditions of transport. The following practices are recommended as per Ministry of Health (MOH) guidelines:

1. *Specimen containers must be waterproof and leak-proof;*
2. *If the specimen container is a tube, it must be tightly capped and placed in a rack to maintain it in an upright position;*
3. *Specimen containers and racks should be placed in robust, leak-proof plastic or metal transport boxes with secure, tight fitting covers;*
4. *The transport box should be secured in the transport vehicle;*
5. *Each transport box should be labeled appropriately, consistent with its contents, and marked "PATHOLOGICAL";*
6. *Specimen data forms and identification data should accompany each transport box;*
7. *A spill kit containing absorbent material, a chlorine disinfectant, a leak-proof waste disposal container and heavy duty reusable gloves should be kept in the transport vehicle.*

4.2. REQUIRED SUPPLIES

The proposed sample transportation system as currently envisioned requires Plasma Preparation Tubes, in order to lengthen the period of stability of plasma samples for viral load testing from 6 hours to 30 hours. This will likely require a formal scientific evaluation of the PPT tubes, to validate that they are effective and acceptable for use in Rwanda.

There will also be some minimal capital costs required to purchase the supplies necessary for transporting infectious substances (TB and Bacteria culture, or sputum samples for MDRTB cases) in the appropriate manner to NRL including triple box packing.

NB. In the mean time, the new equipment required for the proposed transportation system is in procurement process, and equipment such as cooler boxes will be used.

4.3. TRANSPORT PLANNING

It is the responsibility of the sender to ensure the correct designation, packaging, labeling and documentation of all diagnostic specimens. The effective transport and transfer of infectious materials requires good coordination between the sender, the carrier and the receiver to ensure that the material is transported safely and arrives on time and in good condition. Such coordination depends upon well-established communication and a partner relationship between the three parties. All three parties have specific responsibilities to carry out in the transport action effort. Health centers will send their samples to District hospitals and NRL will collect samples from DH using their vehicles.

The sender (District Hospital Laboratory)

1. Makes advance arrangements with the receiver of the specimens, including investigating the need for a Official letter to be signed by an official from MOH
2. Makes advance arrangements with the carrier to ensure:
 - a. That the shipment will be accepted for appropriate transportation
 - b. That the shipment is undertaken by the most direct routing (direct transport if possible), avoiding arrival at weekends;

3. Prepares necessary documentation, including permits, dispatch, and shipping documents;
4. Notifies the receiver of transportation arrangements once these have been made, well in advance of expected arrival time.

The carrier (National carrier selected before, NRL after)

1. Provides the sender with the necessary shipping documents and instructions for their completion;
2. Assists the sender in arranging the most direct routing, and then confirms the routing;
3. Maintains and archives the documentation for shipment and transport;
4. Monitors required holding conditions of the shipment while in transit;
5. Notifies the sender of any anticipated (or actual) delays in transit.

The receiver (NRL)

1. Obtains the necessary authorization (s) from national authorities for the transportation of the material;
2. Provides the sender with the required import permit(s), letter(s) of authorization, or other document(s) required by the national authorities;
3. Provides advice and training to the staff from District about correct packaging;
4. Arranges for the most timely and efficient collection on arrival;
5. Immediately acknowledges receipt to the sender.
6. Shipments should not be dispatched until:
 - a) Advance arrangements have been made between the sender, carrier and receiver
 - b) The receiver has confirmed with the national authorities that the material may be legally transported within the country.
 - c) The receiver has confirmed that there will be no delay incurred in the delivery of the package to its destination.

Requirements for air mail

Infectious substances in Category A will not be accepted for shipment through postal services.

Infectious substances in Category B may be shipped by registered air mail, and the Universal Postal Union recommends the following procedure. NRL will develop a punctual contract when this shipment is needed. The basic triple packaging system is used with the same requirements as for other means of transport. The address label shall display the word “*Lettre*” or “Letter” and the green Customs Declaration Label for Postal Mail is required for international mailing. “BIOLOGICAL SUBSTANCE, CATEGORY B” shall be identified with the white diamond label with black letters “UN 3373” (see Figure 1).



Figure1. Different containers of transport
Spill clean-up procedure

The appropriate response in the event of exposure to any infectious substance is to wash or disinfect the affected area as soon as possible, regardless of the agent. Even if an infectious substance comes into contact with non-intact skin, washing of the affected area with soap and water or with an antiseptic solution can reduce the risk of infection. Medical advice should be obtained any time there is a suspected exposure to infectious substances resulting from a damaged package. The following procedure for clean-up can be used for spills of all infectious substances including blood:

1. Wear gloves and protecting clothing, including face and eye protection if indicated.
2. Cover the spill with a cloth or paper towels to contain it.
3. Pour an appropriate disinfectant over the cloth or paper towel and the immediate surrounding area; (5% bleach solutions are generally appropriate, but for spills on aircraft, quaternary ammonium disinfectants should be used).
4. Apply the disinfectant concentrically beginning at the outer margin of the spill area, working towards the centre.
5. After about 30 min (depending on the disinfectant and concentration used) clear away the materials. If there is broken glass or other sharps are involved, use a dustpan or a piece of stiff cardboard to collect the materials and deposit them into a puncture-resistant container for disposal.
6. Clean and disinfect the area of the spillage (if necessary, repeat steps 2–5).
7. Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.

8. After successful disinfection, report the incident to the competent authority and inform them that the site has been decontaminated (see Incident reporting below).

Incident reporting

The various international mode regulations require the reporting of incidents to the relevant competent transport authorities in addition to the necessary health authorities. This applies to both categories of infectious substances, but particularly to those in Category A.

**** Detailed information on disinfectants and their recommended use can be found in Laboratory bio-safety manual, 3rd ed., Geneva, World Health Organization, 2004.*

5. SCHEDULE OF ACTIVITIES

5.1. PROJECT SCOPE

The proposed sample transportation pilot study includes 15 Hospitals and all their outlying health centers, with dedicated vehicles traveling on routes with an average of 2 District Hospitals per route. The initial pilot covers 3 months and includes the following routes and District Hospitals:

5.2. ITINERARIES

N°	ITINERARIES	NUMBER OF DAYS PER WEEK
1.	Byumba-Rutongo	1
2.	Kibogora-Bushenge	2
3.	Shyira-Ruhengeri	1
4.	Kaduha-Gitwe	1
5.	Gakoma-Nyanza-Kabgayi	1
6.	Nyagatare-Ngarama-Kiziguro-Gahini	1

6. ESTIMATED BUDGET FOR THE PILOT PHASE IN 15 DH (See annex 1)

The expected budget for the proposed pilot sample transportation will be the following:

- Cost of one week: (7 X 84. 960 Frw.) = 594.720 Frw x 1 trips=**594.720 Frw.**

- Cost of one month = **594. 720 Frw X 4= 2.378.880 Frs.**
- Cost of 3 months = **2.378.880 Frw X 3= 7.136.640 Frs.**

The transport will be done once a week for each route. Sites which have significant difficulties in sample transport have been chosen. These include 1 route in West Province, 1 route in East Province, 2 routes in South Province, 1 route in North Province and 1 route combining West and North provinces.

7. PROJECT TIMELINE

The pilot study extends from July 2010 to September 2010. After, the system will be extended to all District Hospitals in the country.

8. ROLES AND RESPONSIBILITIES

8.1. ROLES AND RESPONSIBILITIES FOR NRL

In order to maintain the integrity and accurate processing of samples, the NRL will take the lead in the management of the sample transportation system:

- ✓ The NRL is responsible for providing guidelines that address all requirements for good sample transportation, including packaging and labeling of samples.
- ✓ The NRL will work with all district hospitals to establish a comprehensive pick-up calendar, in order to avoid the overworking of district hospitals.
- ✓ The NRL will train the transport companies concerned how to transport the infectious materials.
- ✓ The NRL will train District Hospitals and health centers how to package infectious samples.

8.2. ROLES AND RESPONSIBILITIES FOR DISTRICT HOSPITALS

- ✓ In order to make appropriate decisions, personnel at District Hospitals must understand their obligation to be familiar with regulatory requirements. Dangerous goods regulations require all personnel involved in transport to undergo appropriate training.
- ✓ The District Hospitals will assure the good collection and storage of samples waiting to be transported to the NRL.
- ✓ The District Hospitals will ensure a good distribution of results from NRL.

8.3. ROLES AND RESPONSIBILITIES FOR HEALTH CENTERS

- ✓ The health centers will ensure a good and on-time collection and transportation of the samples to the District Hospital

- ✓ The health centers must respect the guidelines given by NRL and the calendar of picking up samples in order to keep the integrity and stability of samples.

8.4. ROLES AND RESPONSIBILITIES FOR TRANSPORT COMPANY

- ✓ The transport companies will respect all guidelines described by NRL concerning the transportation of infectious samples in order to avoid some contaminations which can occur during transportation.
- ✓ The transport companies must respect the time of picking up samples from district hospitals in order to respect the stability and viability of samples.

9. ESTIMATED BUDGET FOR NATION WIDE EXPANSION PHASE (See Annex 2)

The expected budget for the proposed sample transportation will be the following:

- Cost of one week: 20 trips x 84. 960 Frw = **1.699. 200 Frw**
- Cost of one year: 1.699.200 X 52 weeks = **88.358.400 Frw.**

10. FUNDING MECHANISM

10.1. POTENTIAL COST SHARING

After sensitization of the importance of the sample transportation system, the budget will be shared with all partners, programs, and government institutions involved in health sector for different activities (HIV, TB, Malaria, Epidemic diseases).

10.2. POTENTIAL SOURCES OF OUTSIDE DONOR FUNDING

In the beginning of the system, the funds will be given by donors interested in this activity, but in order to have a sustainable system, the NRL will determine the budget line for sample transportation in Rwanda Laboratory Network by implementing a sustainable system.

11. LIMITATIONS OF LABORATORY CAPACITY

For NRL, the workload at the reception desk and the process of clearing the plasma for viral load will increase. The administrative burden for District Hospitals to coordinate sample collection and distribution of laboratory results from NRL will also increase. The addition of an intermediary could increase the risk of samples being misplaced. There will also be additional cost in terms of personnel and time associated with a centralized system to receive the specimens from many sites on the same day.

12. GEOGRAPHIC REACH OF PROPOSED SYSTEM

After the pilot phase is completed, the sample transportation system will be expanded to all district hospitals in the country. The following table shows us how District Hospitals and CHUB are grouped.

ITINERARIES

No	ITINERARIES	NUMBER OF DAYS USED
1	Byumba-Rutongo	1
2	Butaro	1
3	Nemba-Gisenyi	1
4	Kabaya	1
5	Remera Rukoma-Muhororo	1
6	Mibilizi-Gihundwe	2
7	Kibogora-Bushenge	2
8	Shyira-Ruhengeri	1
9	Ruli-Nyamata	1
10	Kigeme-Munini	1
11	Kaduha-Gitwe	1
12	Kilinda-Mugonero	2
13	Gakoma-Nyanza-Kabgayi	1
14	Murunda-Kibuye	1
15	Kibilizi-Kabutare-CHUB	1
16	Nyagatare-Ngarama-Kiziguro-Gahini	1
17	Kirehe-Rwamagana-Kibungo-Rwinkwavu	1

13. CONCLUSION

In conclusion, the National Reference Laboratory believes that piloting a new sample transportation system at selected District Hospitals and CHUB throughout the country will have a positive impact on patient outcomes. This pilot phase will also create an operational model to expand on a national scale in 2010, as Early Infant Diagnosis of HIV and viral load testing are expanding.

ANNEX 0: SPECIFICATIONS OF INFECTIOUS SUBSTANCES

(Sources: World Health Organization)

Infectious substances

For the purposes of transport, infectious substances are defined as substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsiae, parasites, fungi) and other agents such as prions, which can cause disease in humans or animals. The definition is applied to all specimens except those explicitly excluded (see below). Infectious substances are divided into two categories:

Infectious substance, Category A

An infectious substance which is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals. Indicative examples of substances that meet these criteria are given in the table in **Annex 2**.

NOTE: An exposure occurs when an infectious substance is released outside of the protective packaging, resulting in physical contact with humans or animals.

(a) Infectious substances meeting these criteria which cause disease in humans or both in humans and animals shall be assigned to United Nations number UN 2814. Infectious substances which cause disease only in animals shall be assigned to UN 2900.

Dangerous goods are assigned UN numbers and proper shipping names according to their hazard classification and their composition. Proper shipping names are used to clearly identify the dangerous article or substance.

(b) Assignment to UN 2814 or UN 2900 shall be based on the known medical history and symptoms of the source human or animal, endemic local conditions, or professional judgment concerning individual circumstances of the source human or animal.

NOTE 1: The proper shipping name for UN 2814 is INFECTIOUS SUBSTANCE, AFFECTING HUMANS. The proper shipping name for UN 2900 is INFECTIOUS SUBSTANCE, AFFECTING ANIMALS only.

NOTE 2: The table in Annex 2 is not exhaustive. Infectious substances, including new or emerging pathogens, which do not appear in the table but which meet the same criteria shall be assigned to Category A. In addition, if there is doubt as to whether or not a substance meets the criteria it shall be included in Category A.

NOTE 3: In the table in Annex 2, the microorganisms written in italics are bacteria, mycoplasmas, rickettsiae or fungi.

Infectious substance, Category B

An infectious substance which does not meet the criteria for inclusion in Category A. Infectious substances in Category B shall be assigned to UN 3373. **NOTE:** The proper shipping name of UN 3373 is “BIOLOGICAL SUBSTANCE, CATEGORY B”.

Cultures

Cultures are the result of a process by which pathogens are intentionally propagated. This definition does not include human or animal patient specimens as defined below. Cultures may be classified as Category A or Category B, depending on the microorganism concerned.

Patient specimens

These are human or animal materials, collected directly from humans or animals, including, but not limited to, excreta, secretions, blood and its components, tissue and tissue fluid swabs, and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Biological products

Biological products are those products derived from living organisms which are manufactured and distributed in accordance with the requirements of appropriate national authorities, which may have special licensing requirements, and are used either for prevention, treatment, or diagnosis of disease in humans or animals, or for development, experimental or investigational purposes related thereto.

They include, but are not limited to, finished or unfinished products such as vaccines.

Genetically modified microorganisms and organisms

Genetically modified microorganisms and organisms are microorganisms and organisms in which genetic material has been purposely altered through genetic engineering in a way that does not occur naturally. Those genetically modified microorganisms and organisms that do not meet the definition of an infectious substance but which are capable of altering animals, plants or microbiological substances in a way not normally the result of natural reproduction shall be assigned to UN 3245 and shipped following Packing Instruction P904 (ICAO/IATA PI913) – this is not considered further in these guidelines.

Medical or clinical wastes

Medical or clinical wastes are wastes derived from the medical treatment of animals or humans or from bio-research. Medical or clinical wastes containing Category A infectious substances shall be assigned to UN 2814 or UN 2900 as appropriate. Medical or clinical wastes containing Category B infectious substances, or which are reasonably believed to have a low probability of containing infectious substances, shall be assigned to UN 3291 and shipped following Packing

Instruction P621

(ICAO/IATA PI622) – **this is not considered further in these guidelines.**

Exceptions

Because of the low hazard they present, the following substances of biological origin are exempted from dangerous goods requirements and regulations:

- Substances that do not contain infectious substances or will not cause disease in humans or animals
- Substances containing microorganisms that are not pathogenic to humans or animals
- Substances in a form in which any pathogens present have been neutralized or inactivated such that they no longer pose a health risk
- Environmental samples (including food and water samples) that are not considered to pose a significant risk of infection
- Blood and/or blood components collected and shipped for the purposes of transfusion and/or Transplantation
- Dried blood spots and faecal occult blood screening tests
- Decontaminated medical or clinical wastes.

Exemptions

Certain patient specimens may be shipped as exempt. However, in such cases, a set of minimal requirements must be followed. The criteria for exemption and the shipping requirements are outlined below.

Exempt Human/Animal Specimens

Human or animal specimens (patient specimens) for which there is minimal likelihood that pathogens are present are not subject to these Regulations if the specimen is transported in a packaging which will prevent any leakage and which is marked with the words “Exempt human specimen” or “Exempt animal specimen”, as appropriate. The packaging should meet the following conditions:

- (a) The packaging should consist of three components:
 - (i) A leak-proof primary receptacle(s);
 - (ii) A leak-proof secondary packaging; and
 - (iii) An outer packaging of adequate strength for its capacity, mass and intended use, and with at least one surface having minimum dimensions of 100 mm × 100 mm;
- (b) For liquids, absorbent material in sufficient quantity to absorb the entire contents should be placed between the primary receptacle(s) and the secondary packaging so that, during transport, any release or leak of a liquid substance will not reach the outer packaging and will not compromise the integrity of the cushioning material;
- (c) When multiple fragile primary receptacles are placed in a single secondary packaging, they should be either individually wrapped or separated to prevent contact between them.

NOTE 1: An element of professional judgment is required to determine if a substance is exempt under this paragraph. That judgment should be based on the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions. Examples of specimens which may be transported under this paragraph include the blood or urine tests to monitor cholesterol levels, blood glucose levels, hormone levels, or prostate specific antibodies (PSA); those required to monitor organ function such as heart, liver or kidney function for humans or animals with non-infectious diseases, or therapeutic drug monitoring; those conducted for insurance or employment purposes and are intended to determine the presence of drugs or alcohol; pregnancy test; biopsies to detect cancer; and antibody detection in humans or animals.

NOTE 2: For air transport, packaging for specimens exempted under this paragraph shall meet the conditions in (a) to (c).

ANNEX 1: DETAILED BUDGET PER SAMPLE TYPE AND DISEASES

Budget of Integrated Sample Transportation System in the Laboratory Network Pilot phase (15 DH)								
Diseases	Sample type	Collected samples	Number of samples		Number of Health facilities to be covered	% per sample type	% per disease	Price per sample type and disease (RWF)
TB	TB	sputum for culture/DST/PCR	157	942	15	5,1%	5,1%	363,969
		QA/QC slides	785		15			
HIV	EID	DBS	1,486	1,486	15	8,1%	90,3%	6, 444,386
	HIV	Viral load	1,221	8,197	15	44,4%		
		serology QA/QC	6,976		15			
	CD4	QA/QC	4,604	4,604	15	25%		
	Hematology & Chemistry	QA/QC	2,372	2,372	15	12,9%		
Influenza	Influenza	Swab	327	327	15	1,8%	1,8%	128,460
Malaria	Malaria	slides QA/QC	473	490	15	2,7%	2,7%	192,689
		DBS	17		15			
Epidemics	Bacteriology	blood, stool, urine, CSF	23	23	15	0,1%	0,1%	7,137
TOTAL				18441				7, 136,641

Budget of Integrated Sample Transportation System in the Laboratory Network per month (43 DH, Expansion Phase)								
Diseases	Sample type	Sample collected	Number of samples		Number of Health facilities to be covered	% per sample type	% per disease type	Price per sample type and diseases (RWF)
TB	TB	Sputum for culture/DST/PCR	150	900	43	5,1%	5,1%	375,523
		QA/QC slides	750		43			
HIV	EID	DBS	1,420	1,420	43	8,1%	90,3%	6, 648,969
	HIV	Viral load	1,167	7,834	43	44,4%		
		serology QA/QC	6,667		43			
	CD4	QA/QC	4,400	4,400	43	25%		
	Hematology & Chemistry	QA/QC	2,267	2,267	43	12,9%		
Influenza	Influenza	Swab	313	313	43	1,8%	1,8%	132,538
Malaria	Malaria	slides QA/QC	452	469	43	2,7%	2,7%	198,806
		DBS	17		43			
Epidemics	Bacteriology	blood, stool, urine, CSF	22	22	43	0,1%	0,1%	7,363
TOTAL				17,625			TOTAL PER MONTH	7, 363,200

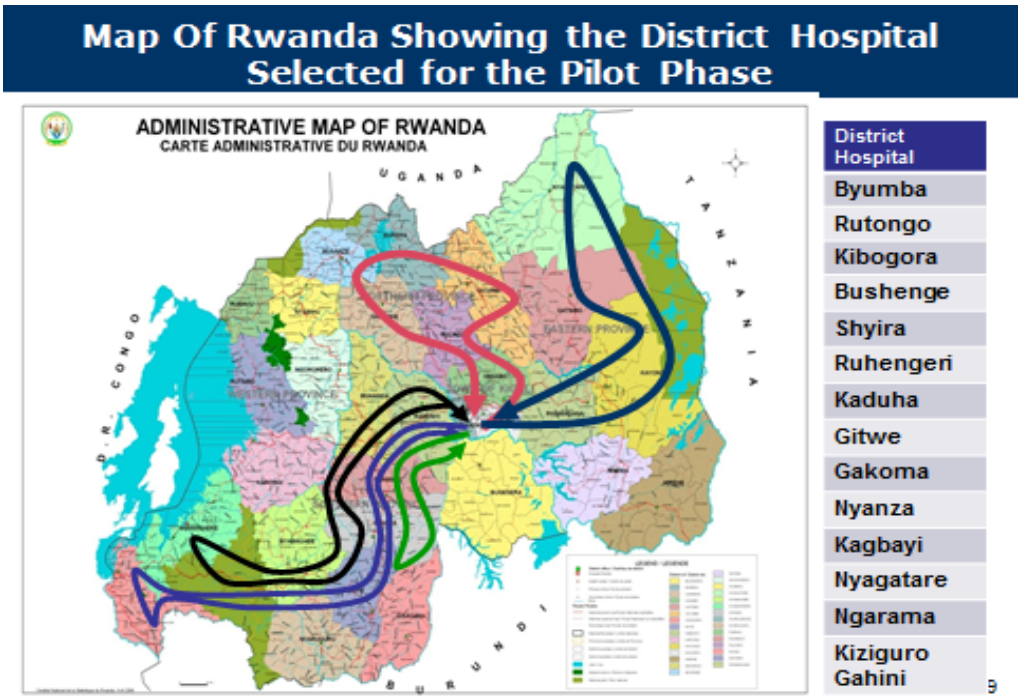
**Budget of Integrated Sample Transportation System in the Laboratory Network per quarter
(43 DH, Expansion Phase)**

Diseases	Sample type	Sample collected	Number of samples		Number of Health facilities to be covered	% per sample type	% per disease	Price per sample type and diseases (RWF)
TB	TB	sputum for culture/DST/PCR	450	2,700	43	5,1%	5,1%	1, 126,570
		QA/QC slides	2250		43			
HIV	EID	DBS	4,260	4,260	43	8,1%	90,3%	19, 946,909
	HIV	Viral load	3,500	23,500	43	44,4%		
		serology QA/QC	20,000		43			
	CD4	QA/QC	13,200	13,200	43	25%		
	Hematology & Chemistry	QA/QC	6,800	6,800	43	12,9%		
Influenza	Influenza	Swab	938	938	43	1,8%	1,8%	397,612
Malaria	Malaria	slides QA/QC	1,357	1,407	43	2,7%	2,7%	596,419
		DBS	50		43			
Epidemics	Bacteriology	blood, stool, urine, CSF	65	65	43	0,1%	0,1%	22,090
			TOTAL samples collected	52,870			TOTAL PRICE PER QUARTER	22, 089,600


Budget of Integrated Sample Transportation System in the Laboratory Network per year (43 DH, Expansion Phase)

Diseases	Sample type	Sample collected	Number of samples		Number of Health facilities to be covered	% per samples type	% per disease	Price per sample type and disease (RWF)
TB	TB	sputum for culture/DST/PCR	1,800	10,800	43	5,1%	5,1%	4, 506,278
		QA/QC slides	9,000		43			
HIV	EID	DBS	17,040	17,040	43	8,1%	90,3%	79, 787,635
	HIV	Viral load	14,000	94,000	43	44,4%		
		serology QA/QC	80,000		43			
	CD4	QA/QC	52,800	52,800	43	25%		
	Hematology & Chemistry	QA/QC	27,200	27,200	43	12,9%		
Influenza	Influenza	Swab	3,752	3,752	43	1,8%	1,8%	1, 590,451
Malaria	Malaria	slides QA/QC	5,428	5,628	43	2,7%	2,7%	2, 385,677
		DBS	200		43			
Epidemics	Bacteriology	blood, stool, urine, CSF	260	260	43	0,1%	0,1%	88,358
			TOTAL samples collected	211,480			TOTAL PER YEAR	88, 358,400

ANNEX2: MAPPING OF SITES TO BE COVERED



ANNEX 3 : STANDARD OPERATING PROCEDURES

 <p>National Reference Laboratory (NRL) Division</p>	Title: Viral Load samples transportation			
	SOP Number: NRL-LAB-PCR-018-VERS01	Effective Date: April 26, 2012	Review Date: April 26, 2013	Pages: 06
	Prepared and signed by: Jean Pierre Musabyimana (Team leader)	Authorised and signed by Head of Division: Dr Odette MUKABAYIRE		

Revision history			
Revised by	Version	Effective date	Description of change
NA	Original	May 26, 2012	NA

Content

1. Background and purpose
2. Definition
3. Scope
4. Responsibilities
5. Equipment and consumables
6. Procedures
7. References

1. BACKGROUND AND PURPOSE

In the interest of public health, human pathological specimens need to be transported safely, timely, efficiently and legally from the place where they are collected to the place where they will be analyzed. Regardless of the presumed infection status of the sample, any specimen of human origin should be packaged and transported in such a way that protects those engaged in transportation from the risk of infection.

The current SOP aims to develop procedures followed while sending specimen for viral load testing from Health Centers to various District Hospitals and from DH to NRL for the purpose of performing essential laboratory tests.

Transportation services will be supplied by public chartered transport companies, which will provide dedicated vehicles to collect samples from various district hospitals, and deliver them to the NRL twice a week. The companies will receive training on the regulations and procedures of sample transportation to NRL.

2. DEFINITIONS AND ACRONYMS

- **Specimen:** is a portion or quantity of material for use in testing, examination, or study.
- **Packaging box:** is a container whereby samples are put in conditions that prevent harm to their nature
- **Triple packaging:** Packaging for all substances should consist of three components (triple packed)
- **Transportation system:** a system whereby samples are collected and sent to the testing laboratory and results thereof from the laboratory to sample expeditor, all in a safe and systematic manner
- **Dedicated vehicles:** A special vehicle able to carry medical specimens.
- **Precautions:** Universal precautions refers to the practice, in [medicine](#), of avoiding contact with patients' bodily fluids, by means of the wearing of nonporous articles such as [medical gloves](#), [goggles](#), and [face shields](#). Under universal precautions all patients are considered to be possible carriers of blood-borne pathogens.
- **Infectious material:** infectious substances are defined as substances which are known or are reasonably expected to contain pathogens.

3. SCOPE OF APPLICATION

This SOP applies to all staff at the NRL, DH and HC that is concerned with sample transportation system. This document should also be read and implemented by all NRL staff, and technologists at DH and HC. The current SOP involves also drivers and their dedicated vehicles that will be taking specimens from one site to another.

4. RESPONSIBILITY

Responsibilities have been assigned as follows:

Task	Person responsible
Ensure that guidelines that address all requirements for good sample transportation, including packaging and labeling of samples are provided.	Head of Immuno-virology Unit
Ensure that all relevant people have read and implemented this SOP, are trained accordingly and this is documented appropriately.	Molecular biology Laboratory In-charge Head of Immuno-virology Unit
Work with all district hospitals to establish a comprehensive pick-up calendar, in order to avoid the overworking of district hospitals.	NRL
Train the transport companies concerned on how to transport the infectious materials.	NRL
Train District Hospitals and health centers how to package infectious samples.	NRL
Packaging	Laboratory biotechnologists at HC and DH
Transportation	Drivers
Results feedback	Molecular Biology Laboratory In-charge Head of Immuno-virology Unit

5. EQUIPMENT AND SUPPLIES

5.1 REAGENTS

- PPT tubes
- Cool boxes

5.2. EQUIPMENT and consumables supplies

- Biohazard box
- PPT Tubes
- Biohazard container

6. PROCEDURES:

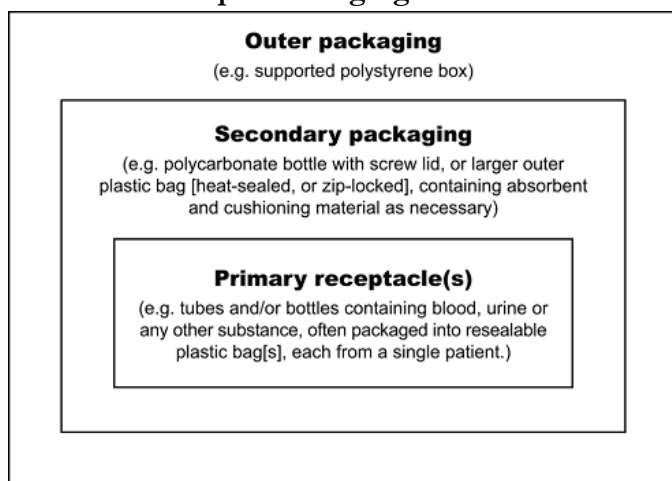
6.1. SPECIAL SAFETY PRECAUTIONS

- Standard precautions applying to the viral load Specimen handling to the current SOP's for Viral Load testing.
- As a consequence, health care providers will be encouraged to wear protective clothing, including gloves, gowns, and masks to protect themselves, the patients or materials, and the environment from infections. When shipped, the specimens will be in safe containers that will not be contaminated on the outside. Thus the shippers do not have to wear protective equipments.

- Each transport box will be labeled appropriately, consistent with its contents, and marked “PATHOLOGICAL”.
- A spill kit containing absorbent material, a chlorine disinfectant, a leak-proof waste disposal container and heavy duty reusable gloves will be kept in the transport vehicle.
- Any other precautions please refer to Biosafety SOP.

6.2. Packaging

6.2.1. Triple Packaging



To fulfill the requirements of triple packaging, note that the common packaging arrangement shown in the above figure. The primary receptacles are placed inside an additional container (which could be as simple as a larger plastic bag with a zip-lock, or a heat sealed plastic bag) containing absorbent material sufficient to absorb any likely spill, before being placed in the outer packaging container.

Primary packaging

- Whole blood will be collected in PPT tubes at Health facilities (DH/HC).
- Maintain the tube in the upright position.

Secondary packaging

- The specimens collected in the PPT tube will be put in a second box at health facility (HC/DH),
- The tube container must be tightly capped and placed in a rack to maintain it in an upright position.
- Send the box to the District Hospital for a third packaging.
- Specimen containers and racks should be placed in robust, leak-proof plastic or metal transport boxes with secure, tight fitting covers.

Thirdly packaging

- The second box will be packaged in the third box at District Hospital site and ready for transport to NRL.
- The transport box will be secured in the transport vehicle.

1.1.1. FREQUENCY OF TRANSPORTATION

Trained drivers will deliver Boxes containing falcon tubes with PPT tubes for viral load testing

• *From HC to DH*

The samples from health centers will continue to be sent to district hospitals by the existing methods (Motorbike, public transport, taxi).

• *From DH to NRL*

The hired vehicles will pick up specimens together with their request form from District Hospitals and deliver them to the NRL once a week.

The request forms will be in a sealed envelop marked “**Confidential**” to keep the confidentiality of any medical information from patients.

Before the introduction of the system, the District Hospital authorities will be briefed and ideas exchanged in order to have a durable and sustainable system.

Specimen data forms and identification data should accompany each transport box in one envelop.

• *Reception of sample specimens at NRL*

The reception at NRL will cross-check the transport boxes with the specimens data forms and record them in the register book. Those that meet the transport conditions will be received and send to molecular biology section for further analyses.

The NRL reception will cross-check the inadequacy and adequacy of VL specimens (see Rejection criteria of inadequate VL specimen).


1.1.2. RESULTS FEED-BACK

- As the drivers go back to Districts Hospitals, the available viral load results will be taken back to the Districts Hospitals.
- Results will be in a sealed envelop and marked as “**Confidential**”
- Health Centres will be called to come and pick up their respect available results from the District Hospitals.
- Drivers will sign for receiving and transportation of both specimens and results feed-back to sites.

NB: Please, note that a specimen collected in the PPT tube for viral load testing should reach at the National Reference Laboratory within the 48 hours

Members of the Team for SOP preparation

1. Musabyimana Jean Pierre
2. Kabalisa Emmanuel
3. Uwimbabazi Jean Claude
4. Dr Serumondo Janvier

 A Healthy People. A Wealthy Nation National Reference Laboratory (NRL) Division	Title: Standard operation procedure for Dry Blood Spot Collection, Handling, Storage and transport			
	SOP Number: NRL-LAB-PCR-002-VERS03	Effective Date: April 20, 2012	Review Date: April 20, 2013	Pages: 12
	Prepared and signed by: J. Claude UWIMBABAZI (Team leader)	Authorised and signed by Head of Division: Dr Odette MUKABAYIRE		

Revision history			
Revised by	Version	Effective date	Description of change
NA	Original	May 26, 2010	NA
J.Claude UWIMBABAZI	VERS02	April 20, 2011	
J.Claude UWIMBABAZI	VERS03	April 20, 2012	P1

1. PURPOSE AND SCOPE

Dried Blood Spots (DBS) are whole blood samples collected on filter paper and dried. They are made directly from the client's whole blood in general and children in particular. DBS are used for HIV Early Infant Diagnosis or re-testing at a reference laboratory, which may be part of External Quality Assurance. DBS samples are useful for HIV EID as they are easy to collect, store, and transport. The purpose of this standard operating procedure is to detail the steps to correctly collect, dry, store, and ship DBS samples.

2. RESPONSIBILITY

It is the responsibility of each staff member (Nurse, Clinician and/or lab biotechnologist) performing the finger stick collection and collecting a dry blood spot (DBS) specimen to adhere to these standard operating procedure. Each staff member performing capillary blood sample collections and performing the DBS collection must review this operating procedure and receive training based on the information and procedures detailed within.

3. BIOSAFETY

Collecting DBS specimens and performing HIV DNA PCR testing poses a potential health hazard. All specimens should be treated as though they are potentially infectious. Therefore, coming in contact with human blood or blood products should be minimized. Personnel protective equipment such as gloves (poderless) and lab coats must be worn at all times when collecting and/or handling DBS samples. Refer to the laboratory safety standard operating procedures for complete laboratory safety practices. Safe collection practices involve taking the

necessary precautions to protect oneself, children and/or client against possible infection as well as DBS samples for any contamination.

4. SPECIMEN REQUIREMENTS

The DBS specimen can be used with whole blood from either a finger stick or a vein. *For the purposes of this standard operating procedure a finger stick will be used to collect a sample of capillary blood for Early Infant Diagnosis. The sample is then transferred directly from the finger to the testing card.*

5. DBS SPECIMEN COLLECTION

DBS specimen collection procedures are described below and additional finger stick equipment and materials are required to perform the DBS collection.

6. PERFORMING THE DRIED BLOOD SPOT (DBS) COLLECTION

Equipment and Materials

- 70 % alcohol or prepackaged 70% alcohol prep-pads
- Dry gauze pads or cotton balls
- Sterile lancets
- DBS collection cards
- Dry rack
- Glycine weighing paper
- Sealable plastic bags
- Humidity cards
- Desiccant packs
- Band-Aids or plasters
- Personal protective equipment (laboratory coat, powder less gloves, etc.)
- Sharps containers
- Biohazard waste receptacles

Procedures

The steps for collecting a DBS sample are as follows:

1. Organize all collection equipment and supplies
2. Describe the procedure to the mother
3. Wash hands and put on gloves (powder less)
4. Using a permanent marker or a pen, record the children identifier on the bottom portion of the correction card, record the collection date and name of the site on the second line of the card.
5. Select the puncture site and clean with 70% ethanol (alcohol).

6. Perform the finger stick and immediately discard the lancet into a sharps container.
7. Wipe away the first drop of blood.
8. Squeeze the finger firmly. Working quickly, hold the filter paper by the edges and touch the filter paper gently against the large drop of blood and in one step allow a sufficient quantity of blood to soak through and completely fill or saturate a circle (spot). A completed saturated spot will contain about 60 µl of blood (one drop of blood).
9. Repeat, until you have collected enough blood to fill at least 2 circles on the blood collection card.
10. Apply pressure to the puncture site using sterile gauze or a cotton ball once at least 2 circles have been collected on the card.
11. Ask the mother to maintain pressure on the site once sample collection is complete.

Additional tips:

- DO NOT press the filter paper against the puncture site.
 - Apply blood to only one side of the filter paper (DBS collection card).
 - Do not layer successive drops of blood or apply blood more than once in the same collection circle.
 - Do not “milk” the finger as excessive milking or squeezing the puncture site might cause haemolysis of the specimen or result in collection of tissue fluids with the specimen, which might adversely affect the test result.
12. Apply a band-aid to the child puncture site if necessary.
 13. Discard any contaminated waste (gauze or cotton balls, etc...) into a biohazard waste receptacle.
 14. Discard material packaging waste into a regular waste container.
 15. Confirm that the patient is okay before allowing the patient to leave or stand.

NOTE: Two complete circles are better than five incomplete ones! While the collection card may include 5 circles, only one child’s blood may be collected on one card.

7. DRYING THE DBS COLLECTION CARD

Procedures

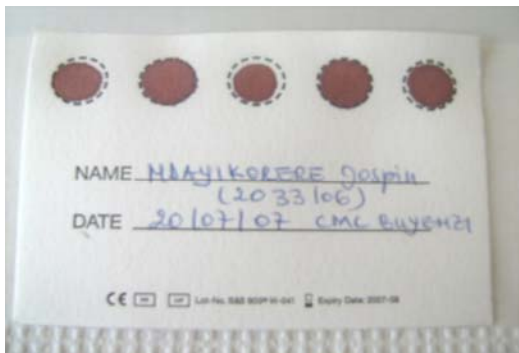
1. Once the specimen has been collected allow the specimen to fully air dry horizontally (at least 3 hours) at room temperature.
2. Avoid touching or smearing the blood spots once they have been collected.
3. Do not heat, stack or allow DBS collection cards to touch other surfaces during the drying process. Use a drying rack or place the cards in position to facilitate drying.

NOTE:

- Keep the DBS samples away from direct sunlight
- Care should be taken to avoid exposing DBS to environmental conditions that may compromise the integrity of the specimen.
- DBS samples should not be dried near an open window as sunlight, dust and in some cases flying insects may come in contact with the DBS during the drying procedure.

7. VALID DBS COLLECTIONS

Provided below is an examples of correctly collected DBS sample.



9. INVALID DBS COLLECTIONS

Provided below are examples of improper DBS collections.

1



This specimen is invalid because quantity of blood is insufficient for testing. This may have been caused by:

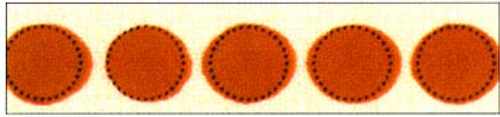
- Removing filter paper before blood has completely filled circle or before blood has soaked through to the other side
- Applying blood to filter paper with a capillary tube
- Filter paper coming in contact with gloved or ungloved hands or substances such as hand lotion or powder, either before or after blood specimen collection

2



This specimen is invalid because it appears scratched or abraded. This may have been caused by applying blood with a capillary tube or other device.

3



This specimen is invalid because the specimen was not dry before mailing. DBS must dry a minimum of 3 hours before packaging and shipping.

4

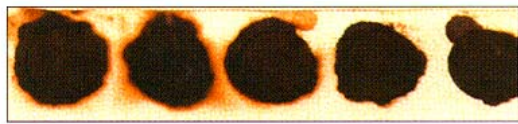


This specimen is invalid because the specimen appears clotted or layered. This may have been caused by:

- Touching the same circle on the filter paper to blood drop several times
- Filling circle on both sides of filter paper

The volume of specimen will not be uniform between spots resulting in errors during the testing process.

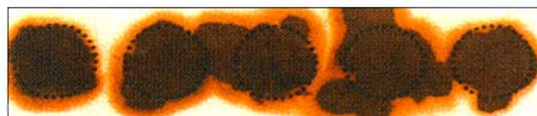
5



This specimen is invalid because the specimen appears hemolyzed, discolored, or contaminated. This may have been caused by:

- Squeezing or “milking” of area surrounding the puncture site
- Allowing filter paper to come in contact with glove or ungloved hands or substances either before or after blood collection
- Exposing blood spots to direct heat

6



This specimen is invalid because the specimen exhibits serum rings – in other words, serum becomes separate from cells. This may have been caused by:

- Not allowing alcohol to dry at puncture site before making skin puncture
- Allowing filter paper to come in contact with alcohol, hand lotion, etc.
- Squeezing area surrounding puncture site excessively
- Drying specimen improperly
- Applying blood to filter paper with a capillary tube

7



This specimen is invalid because no blood was applied to the filter paper.

10. PACKAGING DBS SAMPLES FOR STORAGE

Once the DBS samples have dried they should be packaged for storage until they are ready to be shipped for test.

Procedures

1. Ensure that each DBS is thoroughly dry.
2. Reconcile the Test Request form and check list with all of the DBS specimens that have been collected and dried.
3. Place DBS samples individually into weighing paper in order to prevent cross-contamination
4. Place the stack of DBS samples into the provided sealable plastic bag. Up to 10 DBS samples can be place into each plastic bag. The bag should be just the right size to hold the samples. Avoid using bags that are too big as the DBS will shuffle inside the bag (

1.

NOTE: Sandwich bags will not work. The bag should be a sealable heavy duty plastic bag, one that will prevent moisture from entering and damaging the samples.

5. Add two (at least one) desiccant packets per sample in each bag beside the DBS samples.

NOTE: Do not add desiccant packets into weighing paper containing DBS samples.

6. Add a fresh humidity card into sealable plastic bag.
7. Seal well plastic bag by avoiding air-bibles.
8. Label contents of bag (Number of DBS samples, name of FOSA and partner) with a permanent marker.

11. STORAGE OF DBS SAMPLES

Once DBS samples are packaged in a labeled and sealed plastic bag they must be stored at room temperature in a cool and dry location until they are ready to be shipped. Monitor the color indicator on the humidity cards at frequent intervals if samples are to be stored for a significant length of time. Replace and replenish desiccants and humidity cards as necessary.

- Avoid leaving packaged cards in a vehicle, as sun and heat will deteriorate DBS.
- Avoid placing spots in a malfunctioning refrigerator where water may drip on or soak the spots.

12. SHIPPING DBS SAMPLES

Follow these steps:

1. Ensure that all desiccants and humidity cards are viable. Replace and replenish desiccants and humidity cards as necessary.
2. Label the sealable plastic bag with a biohazard label on the outside indicating bio-hazardous contents
3. Insert the bundled DBS specimens into rip-resistant envelop. Be sure to include appropriate documentation (such as request form & check list) with the shipment.
4. Label the rip-resistant envelop with a permanent marker
5. Ship all DBS samples to the Laboratory for test.

13. DOCUMENTATION

Accurate and complete documentation for each DBS performed is critical to ensure quality of test results. Always record the DBS collection date, child identification, Name of the site (FOSA), Mother 'names, the name of the person requesting or performing the test, test purpose (PCR1, PCR2 or Confirmatory PCR test for Positive rapid test results and on the request form.

Once the test has been done and interpreted, a result will be provided. Send the test results on the original request form received. If deficiencies or discordant results are noted, a corrective action will be required. Maintain all correspondence.

14. REFERENCES

- ✓ UWIMBABAZI JC., “Module de formation pour la collecte, la conservation et le transport du sang sur papier filtre (DBS): Diagnostic précoce du VIH chez l’enfant exposé”, May 2009.
- ✓ CDC, “Blood Collection and Handling - DBS, Trainer’s Guide”, 2005.
- ✓ Richard CK., William ES., Jonathan YR., and Harry HW., “Guideline for shipment of Dried Blood Spot specimens” vol. 16, N° 1, March 9, 1995,

Members of SOP preparation team

1. Jean Claude UWIMBABAZI (Team leader)
2. Dr Alain NYARUHIRIRA
3. Emmanuel KABALISA
4. Richard MWESIGWA NKUNDA

Signing page

We, staff members ofdo hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no.....

We commit ourselves to abide by its spirit and shall strive to comply and make it complied with.

N°	Staff Names	Staff Signature	Date
1			
2			
3			
4			
5			
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I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit / section:

Date:

Amendment Page

Date	Amendment number	Amendment	Page	Section(s) involved	Author

 <p>RWANDA BIOMEDICAL CENTER</p> <p>A Healthy People. A Wealthy Nation</p> <p>National Reference Laboratory (NRL) Division</p>	Title: TB Samples Transportation System			
	SOP Number:	Effective Date:	Review Date:	Pages: 9
	NRL-LAB009-MYC-009AVERS 02	25 April 2012	25 April 2013	
	Prepared and signed by: Uwimana Innocent	Authorised and signed by the Head of Division: Dr Odette MUKABAYIRE		

Revision history			
Revised by	Version number	Effective date	Description of change
Uwimana Innocent	Version 2	25 April 2012	P1 : Replacing the NRL Logo by RBC logo P4 : Primary packaging : Tube with or without CPC P5: Use of present tense instead of future (eg. the second container is packed, the transport box is secured in transport vehicle) P5 : Information given to the driver P6 : Signing form P8 : Signing page

Content

- I. Background and purpose
- II. Definition
- III. Scope
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The current SOP aims to outline procedures followed while sending sputum samples for culture, DST, Rapid PCR test and TB Slides for QA/QC from Health Centers to various District Hospitals the NRL and from DH to NRL for the purpose of performing essential laboratory tests.

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- **Infectious material:** infectious substances are defined as substances which are known or are reasonably expected to contain pathogens.

3. SCOPE OF APPLICATION

This SOP applies to all staff at the NRL, DH and HC (CDT) that is concerned with sample transportation system. This document should also be read and implemented by all NRL staff, and technologists at DH and HC. The current SOP involves also drivers and their dedicated vehicles that will be taking specimens from one site to another.

4. RESPONSIBILITY

Responsibilities have been assigned as follows:

Task	Person responsible
Ensure that guidelines that address all requirements for good sample transportation, including packaging and labeling of samples are provided.	Head of Microbiology Unit
Ensure that all relevant people have read and implemented this SOP, are trained accordingly and this is documented appropriately.	Mycobacteriology Laboratory In-charge Head of Microbiology Unit
Work with all district hospitals to establish a comprehensive pick-up calendar, in order to avoid the overworking of district hospitals.	NRL
Train the transport companies concerned how to transport the infectious materials.	NRL
Train District Hospitals and health centers How to package infectious samples.	NRL
Packaging	Laboratory biotechnologists at HC(CDT), and DH
Transportation	Drivers
Results feedback	Mycobacteriology Laboratory In-charge Head of Microbiology Unit

5. EQUIPMENT AND SUPPLIES

5.1 REAGENTS

- CPC-NaCl: Cetyl Pyridinium Chloride
 - CPC
 - 1M NaCl
- Specimens: TB slides for QA/QC and Sputum

5.2. EQUIPMENT and consumables supplies

- Biohazard box
- Tube falcon
- Biohazard container
- Envelops

6. Procedures:

6.1. SPECIAL SAFETY PRECAUTIONS

- Standard precautions applying to the TB Specimen handling to the current SOP's for TB diagnosis.
- As a consequence, health care providers will be encouraged to wear protective clothing, including gloves, gowns, and masks to protect themselves, the patients or materials, and the environment from infections. When shipped, the specimens will be in safe containers that will not be contaminated on the outside. Thus the shippers do not have to wear protective equipments.
- Each transport box will be labeled appropriately, consistent with its contents, and marked "PATHOLOGICAL".
- A spill kit containing absorbent material, a chlorine disinfectant, a leak-proof waste disposal container and heavy duty reusable gloves will be kept in the transport vehicle.
- Any other precautions please refer to Biosafety SOP.

6.2. Packaging

7.2.1. Triple Packaging

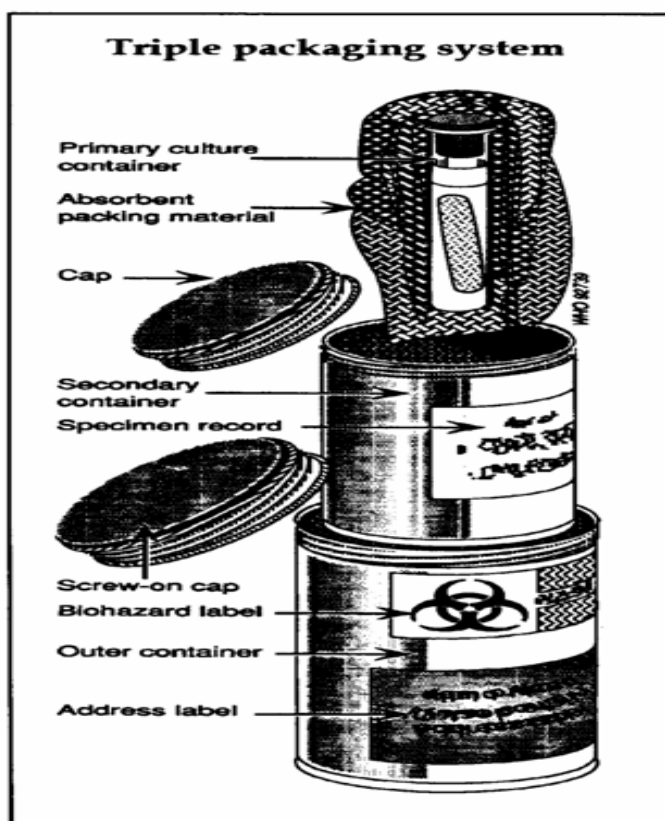


Figure 1: Image showing an example of how triple packaging is done on site.ⁱ

To fulfill the requirements of triple packaging, note that the proper packaging arrangement is shown in the above Figure 1. The primary receptacles are placed inside an additional container (which could be as simple as a larger plastic bag with a zip-lock, or a heat sealed plastic bag) containing absorbent material sufficient to absorb any likely spill, before being placed in the outer packaging container.

Primary packaging

- Sputum is collected in Falcon tube with or without CPC at the CDT/DH.
- Maintain the tube in the upright position.

Secondary packaging

- The specimens collected in the falcon tube with or without CPC will be put in a second box at HC(CDT)/DH,
- The tube container must be tightly capped and placed in a rack to maintain it in an upright position.
- Send the box to the District Hospital for a third packaging.

- Specimen containers and racks should be placed in robust, leak-proof plastic or metal transport boxes with secure, tight fitting covers.

Thirdly packaging

- The second box is packaged in the third box at District Hospital site and ready for transport to NRL.
- The transport box is secured in the transport vehicle.
- The laboratory in charge provides to the driver all information related to the specimens being transported by the vehicle.

7.2.2. FREQUENCY OF TRANSPORTATION

Trained drivers will deliver Boxes containing falcon tubes with sputum for culture and DST and slides for QA/QC a well.

• *From HC (CDT) to DH*

The samples from health centers (CDT) will continue to be sent to district hospitals by the existing methods (Motorbike, public transport, taxi).

• *From DH to NRL*

The hired vehicles pick up specimens together with their request form from District Hospitals and deliver them to the NRL once a week according to the calendar established by NRL.

The requests forms will be in a sealed envelop marked “**Confidential**” to keep the confidentiality of any medical information from patients.

Before the introduction of the system, the District Hospital authorities will be briefed and ideas exchanged in order to have a durable and sustainable system.

Specimen data forms and identification data should accompany each transport box in one envelop.

The driver will sign together with the laboratory in charge, a form containing all information about the type, and number of samples being transported.

• *Reception of sample specimens at NRL*

The reception at NRL will cross-check the transport boxes with the specimens data forms and record them in the register book. Those that meet the transport conditions will be received and sent to Mycobacteriology section for further analyses.

The NRL reception will cross-check the inadequacy and adequacy of TB specimens (see Rejection criteria of inadequate TB specimen).

7.2.3. RESULTS FEED-BACK

As the drivers go back to Districts Hospitals, the available culture and DST results will be taken back to the Districts Hospitals.

Results will be in a sealed envelop and marked as **“Confidential”**

Health Centres will be called to come and pick up their respect available results from the District Hospitals.

Drivers will sign for receiving and transportation of both specimens and results feed-back to sites.

7.3. TB SLIDES FOR QA/QC (See New Directives on EQA)

The TB Slides for QA/QC will be collected by supervisors from District hospitals.

7.3.1. Slide Sampling strategy

Following the interval of sample, the supervisor will collect 15 slides for re-testing at NRL using the formula below:

$$\text{Number..of ..slides.to.be.collected} = \frac{\text{.Nb..of ..slides..tested..in..3months....}}{15}$$

7.3.2. Packaging of slides

The slides will be packaged in appropriate slides holder and are ready to be sent to the National Reference Laboratory for testing.

The results form of the 15 slides is filled according to the results recorded in the TB Log book or register. (Form 1 in annex 4)

The form is put in a sealed envelop and mark **“Confidential”**

8. Signing page

I, staff member ofdo hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no.....


I commit myself to abide by its spirit and shall strive to comply and make it complied with.

N°	Staff Names	Staff Signature	Date
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I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit / section:

Date:

 National Reference Laboratory (NRL) Division	Title : Collection and transport of blood specimen			
	SOP Number:	Effective date:	Revision date:	Pages : 08
	NRL-LAB-BAC 005 Version 03	27 May 2012	27 May 2013	
	Prepared and signed by :		Authorized and signed by the Head of NRL Division:	
	Innocent NZABAHIMANA		Dr Odette MUKABAYIRE	
			Date:	

Revision history			
Revised by	Version number	Effective date	Description of change
Mukankwiro Therese	03	27 May 2012	Logo, Cover page and footer

1. BACKGROUND

Blood specimens may be obtained from patients with pneumonia, meningitis, or fever of unknown origin, among other syndromes.

Pneumonia

Blood cultures will be positive for a bacterial pathogen in approximately 10% – 35% of children with chest x-ray confirmed pneumonia. Because of the time and resources required to collect and process specimens, blood cultures should be obtained from children likely to have bacteremic pneumonia. Pneumonia should be diagnosed using criteria established by the World Health Organization (WHO): if several family members present with the same pneumonic symptoms and / or if wheezing is a major symptom, the etiology is likely to be viral and not bacterial; if the patient is a child under two years of age or a child with fever $>39^{\circ}\text{C}$, bacteremia may be easier to detect.

Meningitis

Blood cultures may be collected from a patient with meningitis when the performance of a spinal tap is contraindicated or when it is not technically feasible.

Fever of unknown origin

Blood cultures collected early after the onset of sustained fever (*i.e.*, suspected typhoid fever) may be positive for *Salmonella* serotype Typhi, a gram-negative bacillus.

2. PURPOSE

This SOP is provided to help ensure appropriate collection of blood sample and subsequent transport to the laboratory by individuals in health facilities or on the field during outbreaks.

3. DEFINITIONS/ ABBREVIATIONS

- SOP: Standard operating Procedure
- NRL : National Reference Laboratory
- DH : District Hospitals

4. SCOPE

This SOP applies to the health facilities staffs involved in collection and transportation of blood samples to the referral laboratories or to the NRL staff involved in outbreak surveillance.

5. RESPONSIBILITIES

Responsibilities have been assigned as follows

Task	Person responsible
Ensure that all relevant people have read this SOP, are trained accordingly and that this is documented appropriately	Director of Microbiology Unit/NRL. Directors of DH Laboratory In-charge, NRL and DH
Ensure proper collection , packaging and transport of samples in order to get good results	Laboratory biotechnologists NRL / DH/HC

6. SUPPLIES

Gloves, syringe, needle, tourniquet, gauze squares, cotton balls, adhesive bandage, puncture resistant container, culture medium and antiseptic; iodine tincture 100 ml of 70% isopropyl alcohol to 1 g of iodine) or povidone-iodine is preferred, but 70% alcohol is an acceptable alternative.

The size of the needle will depend on the collection site and the size of the vein. A 23-gauge needle that is 20 – 25 mm in length or a butterfly needle is generally used for children.

7. PROCEDURES

Collection of blood specimens

Reference laboratories should usually receive isolates, rather than clinical specimens, but blood is a commonly collected clinical specimen, and on which laboratorians should be familiar working.

Infection can be transmitted from patient to staff and from staff to patient during the blood-taking procedure. Viral agents pose the greatest hazard and in some instances are potentially lethal. Of particular importance are the hepatitis viruses and the human immunodeficiency virus (HIV; the virus causing acquired immunodeficiency syndrome [AIDS]).

To decrease the risk of transmission of these viral agents, the following recommendations should be practiced.

- a) Wear latex or vinyl gloves impermeable to liquids.
- b) Change gloves between patients.
- c) Inoculate blood into blood-culture media immediately to prevent the blood from clotting in the syringe. Syringes and needles should be disposed of in a puncture-resistant, autoclavable container. No attempt should be made to recap the needle.

A new syringe and needle must be used for each patient.

- d) Wipe the surface of the blood-culture bottle and the gloves with a disinfectant.
 - e) Label the bottle.
 - f) For the transport to the microbiology laboratory, place the blood-culture medium in a container that can be securely sealed.
 - g) Specimen containers should be individually and conspicuously labeled. Any containers with blood on the outside should be wiped thoroughly. Such containers should be transported in individual, sealed plastic envelopes.
 - h) Remove gloves and discard in an autoclavable container.
 - i) Wash hands with soap and water immediately after removing gloves.
 - j) Transport the specimen to the microbiology laboratory
 - k) In the event of a needle-stick injury or other skin puncture or wound, wash the wound thoroughly with soap and water, encouraging bleeding.
- Report any contamination of the hands or body with blood, or any puncture wound, or any cut to the supervisor and the health service for treatment, as appropriate.

Venipuncture

- a) Gather everything needed to complete the blood collection process (see supplies)

Collecting a large amount of blood from a child can be difficult: 1 – 3 ml is usually sufficient, but volume of blood is directly related to culture yield. Blood cultures from young children should be diluted to 1 – 2 ml of blood in 20 ml of broth (1:10 to 1:20).

Blood cultures from adults should be diluted to 5 – 10 ml of blood in 50 ml of broth (1:5 to 1:10).

b) Select an arm and apply a tourniquet to restrict the flow of venous blood.

c) Vigorously wipe the skin with the 70% alcohol, and swab with the iodine tincture or povidone-iodine. Rub over the selected area. Allow to dry. **If the vein is palpated again, repeat the skin disinfection.**

d) After the disinfectant has dried, insert the needle into the vein with the bevel of the needle face-up. Once the vein is entered, withdraw the blood by pulling back the barrel of the syringe in a slow, steady manner. Air must not be pumped into a vein. After the desired amount of blood is obtained, release the tourniquet and place a sterile cotton ball over the insertion site while holding the needle in place. Withdraw the needle and have the patient hold the cotton ball firmly in place until the wound has stopped bleeding. Inoculate the culture medium. Put the adhesive bandage on the wound.

e) Use vacutainer tubes for blood collection, if they are available. Specimens should be put into a blood-culture bottle immediately and placed in an incubator as soon as possible; if incubation is not feasible, the blood culture bottle can be kept at room temperature (20° – 25°C) for up to 8 hours. Ideally, the blood samples should be processed in a bacteriology laboratory as soon as possible after collection (*i.e.*, within 2 hours).

For the diagnosis of bacterial meningitis, blood should be collected when a spinal tap is contraindicated or cannot be performed for technical reasons.

Transport of blood specimens

Blood cannot be transported before being placed in broth because the collection procedure does not use an anticoagulant. If the blood-culture bottle contains a diaphragm, clean the diaphragm with 70% alcohol and povidone-iodine before inoculating the broth medium.

Alcohol with concentrations greater than 70% has decreased bactericidal activity and should not be used.

a) Inject the blood into the broth culture medium within 1 minute of collection.

The broth culture medium should contain supplemental SPS or haematin to promote survival of any organisms. Swirl the bottle several times. Discard the needle and syringe in a puncture resistant container. Do not re-cap the needle.

Clean the diaphragm of the blood-culture bottle, if necessary. Then label it appropriately with patient identification and the date and time of blood collection.

b) The inoculated medium can be kept at room temperature (20°–25°C) for 4 – 6 hours before incubation at 35°C. Inoculated blood-culture medium must not be placed in a refrigerator.

A portable incubator can be used (temperature range 25°– 35°C).

c) Immediately transport the inoculated media to the laboratory. All inoculated blood-culture media should be received by the laboratory within 12 – 18 hours for subculture and should be protected from temperature extremes (<18°C or >37°C) by using a transport carrier made of, *e.g.*, polystyrene (*e.g.*, Styrofoam), which can keep the samples at moderate temperature.

8. REFERENCES

- WHO Guidelines for the collection of clinical specimens during field investigation of outbreaks (2000)
- WHO, CDC Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing Bacterial Pathogens of Public Health Importance in the Developing World (2003)

Signature page

We, staff members of Bacteriology do hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no NRL-LAB-BAC 005 Vers 02.

We commit ourselves to abide by its spirit and shall strive to comply and make it complied with.

N°	Staff Names	Staff Signature	Date
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I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit / section:

Date:

Amendment Page

Date	Amendment number	Amendment	Page	Section(s) involved	Author

 National Reference Laboratory (NRL) Division	Title: Collection and transport of Cerebrospinal Fluid			
	SOP Number: NRL-LAB-BAC 007 VERS 03	Effective date: 27 May 2012	Revision date: 27 May 2013	Pages :07
	Prepared and signed by : Therese MUKANKWIRO		Authorized and signed by the Head of NRL Division: Dr Odette MUKABAYIRE Date:	

REVISION HISTORY			
Revised by	Version number	Effective date	Description of change
Mukankwiro Therese	03	27 May 2012	Logo, Title, Cover page and footer

1. BACKGROUND

If meningitis is suspected, cerebrospinal fluid (CSF) is the best clinical specimen to use for isolation and identification of the etiologic agent. Suspected agents should include *N. meningitidis*, *S. pneumoniae* and *H. influenza* among others. The collection of CSF should only be performed for diagnosis, by experienced personnel, and under aseptic conditions.

2. PURPOSE

This SOP is provided to help ensure appropriate collection of CSF sample and subsequent transport to the laboratory by individuals in health facilities or on the field during outbreaks.

3. DEFINITIONS/ ABBREVIATIONS

- CSF:
- T-I: Trans- isolate medium

4. SCOPE

This SOP applies to the health facilities staffs involved in collection and transportation of CSF samples to the referral laboratories or to the NRL staff involved in outbreak surveillance.

5. RESPONSIBILITIES

Responsibilities have been assigned as follows:

Task	Person responsible
Ensure that all relevant people have read this SOP, are trained accordingly and that this is documented appropriately	Director of Microbiology Unit/NRL .Directors of DH Laboratory In-charge, NRL and DH
Ensure proper collection , packaging and transport of samples in order to get good results	Laboratory biotechnologists NRL / DH/HC

6. SUPPLIES

The kit for collection of CSF:

- Skin disinfectant
- Sterile gauze and adhesive bandages
- Lumbar puncture needles: 22 gauge/3.5" for adults; 23 gauge/2.5" for children
- Sterile screw-cap tubes
- Syringe and needle
- Transport container
- Trans-Isolate (T-I) medium (if CSF cannot be analyzed in the microbiology laboratory immediately)

7. PROCEDURES

Cerebrospinal fluid (CSF) collection

Usually, three tubes of CSF are collected for chemistry, microbiology, and cytology.

If only one tube of fluid is available, it should be given to the microbiology laboratory. If more than one tube (1-ml each) is available, the second or third tube should go to the microbiology laboratory

Because the presence of blood can affect cultures of CSF, it is suggested that **if more than one tube of CSF is collected from a patient**, the first tube collected (which could contain contaminating blood from the lumbar puncture) **not** be the tube sent to the microbiology laboratory.

Lumbar puncture and cerebrospinal fluid (CSF) transport

The kit for collection of CSF should contain the following items:

- Skin disinfectant
- Sterile gauze and adhesive bandages
- Lumbar puncture needles: 22 gauge/3.5" for adults; 23 gauge/2.5" for children
- Sterile screw-cap tubes
- Syringe and needle

- Transport container
- Trans-Isolate (T-I) medium (if CSF cannot be analyzed in the microbiology laboratory immediately)

Patients should be kept motionless for the lumbar puncture, either sitting up or laying on the side, with the back arched forward so that the head almost touches the knees during the procedure. Disinfect the skin along a line drawn between the crests of the two ilia with 70% alcohol to clean the surface and remove debris and oils, then apply a tincture of iodine or povidone-iodine and let it dry.

Introduce the needle, and collect the drops of fluid (1 ml minimum; 3–4 ml, if possible) into sterile, screw-cap tubes. Label the specimen with patient identification and the date and time of CSF collection.

Transport of CSF specimens

As soon as the CSF has been collected, it should be transported to the microbiology laboratory, where it should be examined as soon as possible (preferably within 1 hour from the time of collection); hand-carry the specimen to the laboratory whenever feasible. **Do not refrigerate the CSF specimen or expose it to extreme cold, and do not expose it to excessive heat or sunlight.** If *N. meningitidis* is suspected to be the cause of the illness and a delay of several hours in processing specimens is anticipated, incubating the CSF (with screw-caps loosened) at 35°C in a 5% CO₂ atmosphere (*i.e.*, in a CO₂-incubator or a candle jar) may improve bacterial survival.

If same-day transport to the laboratory is not possible, CSF should be inoculated aseptically into a Trans-Isolate (T-I) medium with a syringe and then held overnight at 35°C.

T-I medium is a biphasic medium that is useful for the primary culture of meningococci and other etiological agents of bacterial meningitis from CSF; it can be used as a growth medium as well as a holding and transport medium.

8. REFERENCES:

- WHO Guidelines for the collection of clinical specimens during field investigation of outbreaks (2000)
- WHO, CDC Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing Bacterial Pathogens of Public Health Importance in the Developing World (2003)

Signature page

We, staff members of Bacteriology do hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no NRL-LAB-BAC 007 VERS 02

We commit ourselves to abide by its spirit and shall strive to comply and make it complied with.

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I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit / section:

Date:

Amendment Page

Date	Amendment number	Amendment	Page	Section(s) involved	Author

 RWANDA BIOMEDICAL CENTER A Healthy People. A Wealthy Nation National Reference Laboratory (NRL) Division	Title: Collection and transport of stool specimen			
	SOP Number:	Effective date:	Revision date:	Pages : 08
	NRL-LAB-BAC 006 VERS 03	27 May 2012	27 May 2013	
	Prepared and signed by :		Authorized and signed by the Head of NRL Division:	
	Innocent NZABAHIMANA		Dr Odette MUKABAYIRE	
			Date:	

REVISION HISTORY			
Revised by	Version number	Effective date	Description of change
Mukankwiro Therese	03	27 May 2012	Logo, Cover page and footer

1. BACKGROUND:

During an outbreak, stool specimens or rectal swabs should be collected from 10–20 persons who meet the following criteria:

- Currently have watery diarrhea (cholera) or bloody diarrhea (dysentery)
- had onset of illness <4 days before sampling; and,
- have not received antimicrobial treatment for the diarrheal illness.

Fecal specimens should be collected in the early stages of any enteric illness, when pathogens are usually present in the stool in highest numbers, and before antibiotic therapy has been started. An exception to this rule is when stool is collected from persons with febrile illness: in the case of typhoid fever, the etiologic agent *Salmonella* ser. Typhi may be present in highest numbers in stool in the second and third weeks of the disease.

2. PURPOSE:

This SOP is provided to help ensure appropriate collection of stool sample and subsequent transport to the laboratory by individuals in health facilities or on the field during outbreaks.

3. DEFINITIONS/ ABBREVIATIONS:

APW: Alkaline peptone water

Carry-Blair: transport medium for stool samples

4. SCOPE:

This SOP applies to the health facilities staffs involved in collection and transportation of stool samples to the referral laboratories or to the NRL staff involved in outbreak surveillance.

5. RESPONSIBILITIES:

Responsibilities have been assigned as follows:

Task	Person responsible
Ensure that all relevant people have read this SOP, are trained accordingly and that this is documented appropriately	Director of Microbiology Unit/NRL .Directors of DH Laboratory In-charge, NRL and DH
Ensure proper collection , packaging and transport of samples in order to get good results	Laboratory biotechnologists NRL / DH/HC

6. EQUIPMENT AND SUPPLIES:

6.1. EQUIPEMENTS:

- A cold box with dry ice

6.2. CONSUMMABLES:

- Clean, dry, leak-proof screw cap container
- Carry- Blair transport media or another appropriate bacterial transport media
- Cotton swabs, Indelible marker, Gloves, and Patient's form

7. PROCEDURES:

Stools samples should be collected in clean containers without disinfectant or detergent residue and with tight-fitting, leak-proof lids. Specimens should not be collected from bedpans, because the bedpans may contain residual disinfectant or other contaminants. **Unpreserved stool should be refrigerated, if possible, and processed within a maximum of 2 hours after collection. Specimens that cannot be cultured within 2 hours of collection should be placed in transport medium and refrigerated immediately.**

Transport media for fecal specimens

This section provides information regarding media appropriate for the transport of fecal specimens that are suspected to contain *Shigella*, *Vibrio cholerae*, or *Salmonella* (including serotype Typhi) specimens. Once specimens from an outbreak of diarrheal disease have arrived at the laboratory, laboratorians should follow procedures for *Shigella* or *V. cholerae* isolation (Appendix 10) depending on whether reports from the field indicate the outbreak appears to be dysentery or a cholera-like illness.

Because persons suspected of having typhoid will commonly present with fever and not diarrhea, laboratories usually do not receive a surge of fecal specimens in an outbreak of typhoid; however, on occasion fecal specimens may be submitted to a laboratory for diagnosis of infection with *S. Typhi* (see Appendix 10 for isolation methods).

Collection and transport of fecal specimens for laboratory diagnosis

When to collect	When the patient is having diarrhea, as soon after onset of illness as possible (preferably within 4 days of onset) and before antimicrobial treatment is started.
How much to collect	Rectal swab or swab of fresh stool in transport medium.
Transport medium	Cary-Blair or other suitable transport medium (NOT buffered glycerol saline for <i>V. cholerae</i>).
Storage after collection	Refrigerate at 4°C if the specimens will be received by the laboratory within 48 hours or freeze at -70°C. Fecal specimens from patients with suspected cholera can be transported at ambient temperature and held for longer times if necessary; however, refrigeration is preferred.
Transportation	Seal tubes/containers to prevent leakage; place in waterproof container to protect from wet or dry ice. Ship in insulated box with ice packs, wet ice, or dry ice by overnight delivery.

Cary-Blair transport medium

Cary-Blair transport medium can be used to transport many bacterial enteric pathogens, including *Shigella*, *Salmonella*, and *Vibrio cholerae* (Figure 81). Cary-Blair's semisolid consistency provides for ease of transport, and the prepared medium can be stored after preparation at room temperature for up to 1 year. Because of its high pH (8.4), it is the medium of choice for transport and preservation of *V. cholerae*.

Other transport media

Other transport media that are similar to Cary-Blair are Amies' and Stuart's transport media. Both of these are acceptable for *Shigella* and *Salmonella* (including ser. Typhi), but they are inferior to Cary-Blair for transport of *V. cholerae*. Alkaline peptone water may be used to transport *V. cholerae*, but this medium is inferior to Cary-Blair and should be used only when the latter medium is not available.

Alkaline peptone water should not be used if subculture will be delayed more than 6 hours from the time of collection, because other organisms will overgrow vibrios after 6 hours.

Buffered glycerol saline (BGS), a transport medium that is used for *Shigella*, is unsuitable for transport of *V. cholerae*. Additional disadvantages of buffered glycerol saline are that it can be used for only 1 month after it is made and, because it is a liquid medium, it is more likely to leak or spill during transport.

FIGURE 81: Cary-Blair semisolid transport medium



Leave the swab in the tube after inoculating Cary-Blair medium.

Placing stool in transport medium

If possible, chill the transport medium for 1–2 hours in a refrigerator or cold box prior to use. A small amount of stool can be collected by inserting a sterile cotton or polyester-tipped swab into the stool and rotating it. If mucus and shreds of intestinal epithelium are present, these should be sampled with the swab.

Following sampling of the stool on the swab:

- a) Insert the swab containing fecal material into transport medium immediately.
- b) Push the swab completely to the bottom of the tube of transport medium.
- c) Break off the top portion of the stick touching the fingers and discard it.
- d) Replace the screw cap on the tube of transport medium and tighten firmly.
- e) Place the tube in a refrigerator or cold box.

8. REFERENCES:

- WHO Guidelines for the collection of clinical specimens during field investigation of outbreaks (2000)
- WHO, CDC Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing Bacterial Pathogens of Public Health Importance in the Developing World (2003)

Signature page

We, staff members of Bacteriology do hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no NRL-LAB-BAC 006 Vers 02

We commit ourselves to abide by its spirit and shall strive to comply and make it complied with.

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I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit / section:

Date:

Amendment Page

Date	Amendment number	Amendment	Page	Section(s) involved	Author

 A Healthy People. A Wealthy Nation National Reference Laboratory (NRL) Division	Title : Malaria EQA(Retesting) Slides collection and transportation System		
	SOP Number: NRL-LAB-PAR003 VERS 03	Effective Date: 26 April 2012	Pages : 5
	Prepared and signed by: Severina MUNYESHYAKA (Team leader)	Authorized and signed by the Head of NRL Division : Dr Odette MUKABAYIRE	

Revision history		
Version number :	Effective date	Description of change
Original	April 2012	NA

PRESERVATION AND DISPATCH OF SAMPLES FOR EXTERNAL QUALITY ASSURANCE

1.1. Purpose

This SOP describes the correct procedure of collection, preservation and proper transportation of the blood smears to the laboratory under optimum conditions for malaria Microscopy.

1.2. Principle

Effective diagnosis depends upon the correct procedure and the time of collection as regard to the stage of the disease, preservation and proper transportation of the clinical samples to the laboratory under optimum conditions.

1.3 Preservation of the blood films at peripheral level

- place the blood films properly in order to allow the thick film to dry evenly.
- protect from flies and dust. When the thick film is completely dry, store the slides in the plastic slide box.
- When long storage is unavoidable, and then keep the slides in a dry cool place away from direct sunlight or any source of heat.
- While storing, place the slides horizontally, which allows the thick film to dry with

even thickness.

4. Malaria SLIDES FOR QA/QC

The Malaria Slides for QA/QC will be collected by supervisors from National reference laboratory.

5. Slide sampling strategy

Count the total number of slides tested in 3 months from the log book

1. Identify and count negative and positive slides separately.
2. Calculate the sample size for positive and negative slides: 20% for positive slides and 2% for negative slides.

6. Packaging of slides

The slides will be packaged in appropriate slides Boxes and are ready to be sent to the National Reference Laboratory for retesting.

The results form is filled according to the results recorded in the malaria Log book or register.

The forms are put in a sealed envelope and marked “**Confidential**”

7. Dispatch of the blood smear from the periphery

- keep the blood smears collected in the field in the slide box till these are

Deposited to the NRL Parasitology section

- wrap these dry slides and use plan paper and avoid mixing up of the slides with those submitted by others and deposit in the laboratory at Parasitology section.

Care should be taken while packing by avoiding 2 smears keeping together.

As both the smears would get attached and both the smears would be lost / damaged.

8. Receipt of the blood smears in the NRL /parasitology section

The Laboratory Technologist will receive these slides and their form.

Laboratory Technologist not leaves the examined slides as such as:

From these slides, remove the oils by gently rubbing with a tissue paper or plan paper

☐ Separate the positive and negative slides and pack separately.

Send these slides for cross checking as per instructions, to the competent Authority.

Do not discard the batch of slides till the cross checking results are received, as Some slides may be required to be re-examined after getting the cross checking Results.

Parasitology Section will ensure that all these procedures are observed in all District Hospital Laboratories.

7. Signing page

We, staff members of Parasitology do hereby certify that we have read, discussed (where applicable) and understood the content of this SOP no: NRL-LAB-PAR 003 Vers 03

We commit ourselves to abide by its spirit and shall strive to comply and make it complied with.

N°	Staff Name	Staff Signature	Date
1	RUZINDANA Emmanuel		
2	TWAHIRWA Moris		
3	MUNYANEZA Tharcis		
4	Severina MUNYESHYAKA		
5	MUCACA J.Bosco		

I,being the head of unit / section indicated above, do hereby certify that all the staff, as listed above, have read, discussed and understood the SOP as indicated herein.

Name and signature of head of unit.

Date:

ANNEX 4. REQUEST AND RESULT FORMS

**BON D'EXAMEN DE LABORATOIRE /CULTURE ET DST**

District Fosa Service..... Date :									
Nom et prénom du patient: Age : <input type="checkbox"/> M <input type="checkbox"/> F									
Adresse : District:..... Secteur: Cellule:..... Umudugudu N° tel									
Nature de l'échantillon <input type="checkbox"/> P <input type="checkbox"/> EP spécifier :							Date prélèvement :		
Examen demandé	<input type="checkbox"/> Bacilloscopie <u>Motif</u> : <input type="checkbox"/> Diagnostic Patient suspecté par <input type="checkbox"/> CDT <input type="checkbox"/> CT <input type="checkbox"/> AS <input type="checkbox"/> Contrôle : <input type="checkbox"/> C2 <input type="checkbox"/> C3 <input type="checkbox"/> C4 <input type="checkbox"/> C5 <input type="checkbox"/> C6 <input type="checkbox"/> C7 <input type="checkbox"/> C8								
	<input type="checkbox"/> Test VIH								
	<input type="checkbox"/> Culture <input type="checkbox"/> DST 1^{ère} ligne <input type="checkbox"/> DST 2^{ème} ligne <input type="checkbox"/> Test rapide (résistance) <u>Malade enregistré comme</u> : <input type="checkbox"/> NTPM+ <input type="checkbox"/> Echec <input type="checkbox"/> Rechute <input type="checkbox"/> Abandon <input type="checkbox"/> TPM- <input type="checkbox"/> TEP <input type="checkbox"/> Autre <input type="checkbox"/> TBMR <u>Contact</u> d'un cas TBMR confirmé : <input type="checkbox"/> Oui <input type="checkbox"/> Non <u>Traitement actuel</u> <input type="checkbox"/> Primotraitement <input type="checkbox"/> Retraitement <input type="checkbox"/> 2 ^{ème} ligne TBMR <input type="checkbox"/> Aucun (suspect de TB) <u>Date début traitement actuel</u> : <u>N° patient dans registre TB</u>								
Nom et signature du demandeur Tél									
Résultats	N° de série Technique : <input type="checkbox"/> Ziehl <input type="checkbox"/> Fluorescence								
	Microscopie	Date de l'échantillon	Echantillon	Apparence <i>(Mucopurulent Salive, Sang)</i>	Nég	1-9	+	++	+++
			1						
			2						
			3						
Culture	N° Technique : <input type="checkbox"/> Lowenstein <input type="checkbox"/> Culture liquide								
	Nég	(1 - 9)	(+)	(++)	(+++)	Contaminé	Pas de colonies		Nég
							< 10 colonies		Nombre exact
							10 -100 colonies		(+)



National Reference Laboratory

FICHE DE TRANSMISSION DES RESULTATS DE DBS

No	Destination	Quantité	Codes and Observations
1			
2			
3			
4			
5			
9			
13			
14			
15			

Pour livraison

Pour réception

Nom & Prénom

Nom & Prénom

Pour transporteur

Nom & Prénom

B. P. 4668, Kigali – RWANDA.

Tel.: (+250) 570414/570400/570399



Laboratoire National de Référence

Code du laboratoire:

FICHE DE DEMANDE D'EXAMEN BACTÉRIOLOGIQUE

Partie demandeur:

Identification du patient :		
Nom : Prénom : Age : Sexe :		
Province : District administratif : Secteur :		
Hôpital : Centre de Santé :		
Type d'échantillon :		
Date et heure de prélèvement :		
Aspect macroscopique :		
Signes cliniques :		
Diagnostic suspecté :		
Prise des médicaments :	OUI : <input type="checkbox"/>	NON: <input type="checkbox"/>
Examens faits par l'hôpital ou C.S. :		
Résultat de l'HD ou C.S. :		
Téléphone de contact :		

Partie LNR:

Date de réception :																																								
Milieu de transport :																																								
Conditions de transport de l'échantillon : Adéquates : <input type="checkbox"/> Inadéquates : <input type="checkbox"/>																																								
<p>Résultats du LNR</p> <p>Culture et Identification :</p> <p>Antibiogramme :</p> <table border="1" style="margin-left: auto; margin-right: auto; border-collapse: collapse;"><thead><tr><th style="width: 50%;">Antibiotiques</th><th style="width: 12.5%;">S</th><th style="width: 12.5%;">I</th><th style="width: 12.5%;">R</th></tr></thead><tbody><tr><td>1</td><td></td><td></td><td></td></tr><tr><td>2.</td><td></td><td></td><td></td></tr><tr><td>3.</td><td></td><td></td><td></td></tr><tr><td>4.</td><td></td><td></td><td></td></tr><tr><td>5.</td><td></td><td></td><td></td></tr><tr><td>6.</td><td></td><td></td><td></td></tr><tr><td>7.</td><td></td><td></td><td></td></tr><tr><td>8.</td><td></td><td></td><td></td></tr><tr><td>9.</td><td></td><td></td><td></td></tr></tbody></table>	Antibiotiques	S	I	R	1				2.				3.				4.				5.				6.				7.				8.				9.			
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8.																																								
9.																																								

Date de remise des résultats :

Signature et Visa du LNR :



Formation Sanitaire : _____	Financement : _____
District Sanitaire : _____	Province : _____
Nom du Bébé : _____	Code Bébé* <table border="1" style="display: inline-table; width: 150px; height: 20px;"></table>
Date de Naissance (jj/mm/aa) : <table border="1" style="display: inline-table; width: 100px; height: 20px;"></table> / <table border="1" style="display: inline-table; width: 100px; height: 20px;"></table> / <table border="1" style="display: inline-table; width: 100px; height: 20px;"></table>	Sexe: M <table border="1" style="display: inline-table; width: 30px; height: 20px;"></table> F <table border="1" style="display: inline-table; width: 30px; height: 20px;"></table>
Nom de la Mère : _____	Code PTMIE <table border="1" style="display: inline-table; width: 150px; height: 20px;"></table>
	TIRA Cnet <table border="1" style="display: inline-table; width: 150px; height: 20px;"></table>

Prophylaxie ARV Mère : AZT + (DU-NVP) DU-NVP Une Semaine (AZT/3TC) Trithérapie AZT/3TC/NVP D4T/3TC/NVP Trithérapie prophylactique (>34 sem.) Autres : _____

Prophylaxie ARV Bébé : _____

Date de Prélèvement (jj/mm/aa) : /

☐ Premier Test PCR (PCR 1) ☐ Allaitement maternel seul ☐ Allaitement artificiel seul

☐ Confirmation du Diagnostic PCR Antérieur (PCR 2) ☐ Allaitement mixte

☐ Confirmation PCR de la sérologie positive à 9 - 18 mois (S) Age de début diversification alimentaire (mois) :

Age à l'arrêt total de l'allaitement (mois) :

Tableau clinique du Bébé : ☐ Symptomatique ☐ Asymptomatique

Cachet et Signature FOSA : Contact Tél. :

Date de Réception au Labo / Code Labo:

Résultat : ☐ POSITIF ☐ NEGATIF ☐ INDETERMINE

<input type="checkbox"/>	Problèmes techniques au Labo
<input type="checkbox"/>	Mal étiqueté
<input type="checkbox"/>	Pas suffisamment de Sang
<input type="checkbox"/>	Autres (Spécifier) :

*1. Code bébé= initiales nom de la FOGA (1er lettre+2 dernières lettres) + code PTME mère + date naissance bébé. Exemple: MDA-0013/10/07. 10/04/07 c'est-à-dire MDA=Murunda hôpital, code mère = numéro d'ordre 0013 du mois d'Octobre 2007; date naissance bébé=10 avril 2007