

Two Person Protocol for Minimal-Exposure Sampling of Wildlife Carcasses

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Introduction

Ebola virus disease (EVD), formerly Ebola hemorrhagic fever (EHF) has achieved particular notoriety in Africa due to its high human case fatality rate (up to 90%), rapid spread in environments with limited health infrastructure and lack of a specific treatment or cure. An effective, experimental preventive vaccine has been developed but is not yet commercially available. Between 1998 and 2013 *Zaire ebolavirus* (EBOV) epidemics resulted in more than 1,000 human deaths and in 2014 EVD (again EBOV) emerged for the first time in West Africa, claiming over 11,000 human lives and reaching pandemic status. Concurrently in August 2014, the Democratic Republic of Congo (DRC) experienced yet another EVD epidemic, followed by an outbreak in 2017 in Bas-Uele and in 2018, in Bikoro and North-Kivu, respectively. EBOV is also highly pathogenic for African great apes (gorillas and chimpanzees). Estimates of EVD-associated ape mortality in central Africa to date is in the tens of thousands, and some gorilla and chimpanzee populations are believed to have been reduced by up to 95% (Walsh et al. 2003; Leroy et al. 2004; Rouquet et al. 2005; Bermejo et al. 2006). EVD has become a major concern for the future viability of African ape populations and was a driving force behind the reclassification of the western lowland gorilla as “critically endangered” by the International Union for the Conservation of Nature (IUCN) (Walsh et al. 2008). Human EVD outbreaks in central Africa have often been preceded by those in wildlife, particularly great apes, and have often been traced to direct contact with great ape carcasses (Leroy et al. 2004).

Carcasses and WCS’s Ebolavirus research: Since the 2005 EVD epidemic in the Republic of Congo (RoC), the Wildlife Conservation Society’s (WCS) Wildlife Health Programme (WHP) has been conducting monitoring and surveillance of great ape and other wildlife populations, as well as targeted research into the ecology of Ebolavirus. The goals of this work are to determine the overall impact of EVD on great ape populations and ecological factors that affect that impact, with the ultimate goal of identifying potential mitigation strategies to protect wild ape populations. Key to this work is early identification of EVD outbreaks in wildlife. Since symptomatic animals are almost never seen, such an outbreak is usually signaled by the presence of wildlife carcasses. Testing carcasses for Ebola virus and other pathogens has to date been our only means of confirming an EVD outbreak in wildlife. To do that, carcasses must be fresh enough to allow collection of usable samples; if too decomposed, the virus will no longer be detectable in the tissues, leading to a false negative test result.

Protecting human health: Since EVD epidemics in this region have invariably been traced to contact with wildlife – usually ape carcasses – the other aim of this initiative is to change behaviors, such as eating wildlife carcasses, that put people at high risk of Ebolavirus exposure. Rural village communities have been educated about the nature of Ebola virus, the serious health risks it poses and how the individuals can protect themselves from contracting it from wildlife. The vast majority of these community members have also been recruited to voluntarily report ape and other wildlife carcasses to both local authorities and WCS. WCS veterinary teams or trained volunteers then collect specimens from those carcasses for diagnostic testing for the detection of Ebola virus. As the Congolese Ministry of Health (via the National Public Health Laboratory) is kept informed throughout the process, this wildlife surveillance tool also acts as an “early-warning system” for human health. It is recognized by the Congolese government in the form of a Letter of Agreement with the Congolese National Public Health Laboratory (NPHL) and is included in the RoC’s Ebola Response Strategy. The network has already proven effective in detecting wildlife mortality and obtaining diagnostic specimens important to both Ebola virus ecology studies and protection of

human health. Just as importantly, the program assists in educational outreach and community engagement, which is crucial to tailoring an effective epidemic response.

Principles of Minimal-Exposure Carcass Sampling

Sampling wildlife carcasses carries with it inherent risks. If the animal died of an infectious disease, the sampler may be exposed to, and risks becoming infected, with that pathogen. However, if appropriate precautions are taken, those risks can be greatly minimized. Every aspect of this Minimal-Exposure Carcass Sampling Protocol is designed to do just that. Though most carcasses test negative for serious pathogens (e.g. Ebola virus, anthrax), every carcass should be treated as though it is potentially infective.

As well as having a calm and positive attitude, there are several basic principles that must be followed:

- **Minimally invasive:** The greater the degree of contact with a potentially infected carcass, the greater the risk of becoming infected. For that reason we have minimal contact with the carcass. Instead of opening a carcass, as is done during a necropsy (animal autopsy), the sampler takes only enough of a sample – in this case fluid collected on swabs – to run the necessary diagnostic testing.
- **Redundant protection:** The principle of redundant protective measures is key to any good biosafety protocol. Building redundancy into the system provides a safe working environment. Each step of this Minimal-Exposure Carcass Sampling Protocol is designed to protect the sampler from contamination. Multiple levels layers of security have built-in redundancy, providing multiple layers of protection, including:
 - **PPE** (water-proof Tyvek™ suit, N95 mask, eye protection, nitrile gloves); a **“buddy system”**; **clean/dirty zones & designated roles**; **triple gloving**; **triple bagging**; **liberal use of disinfectant**; a **clear and concise protocol**.
- **Clean-Dirty:** An additional layer of safety is the Clean/Dirty system of roles and working zones, separated by a “Red Line”. The “buddy system” and designated roles provide a second set of eyes to oversee that procedures are followed, help share the workload, ensure proper execution of the critical donning and doffing of PPE and that there is always one person who can safely handle clean objects and provide disinfection.
- **Triple packaging:** The principle of triple packaging any potentially hazardous materials, including samples and waste, is integral to this carcass sampling protocol. Samples contained in crush-resistant HDPE plastic Nalgene® tubes are placed in three successive zip-closure plastic bags and inside a leak-proof plastic transport bottle.
- **Disinfection, disinfection, disinfection:** The disinfectant that is used is sodium hypochlorite, or common bleach, diluted to a 5% solution. It is highly effective in killing pathogens, including Ebola virus, and is inexpensive and readily available. It is your backup to your PPE and safety protocols. Use it. It never hurts to disinfect something, even a second time. If, at any time, you are concerned about contamination of materials, such as gloves, or are unsure if you already

disinfected a particular item, simply disinfect it again. Better to use too much than too little. Just remember to leave enough for all steps of the protocol.

NOTE: *Sodium hypochlorite is an irritant and is harmful or fatal if ingested. Avoid contact with mucous membranes, such as the eyes and mouth.*

- **Communication:** Communication between the Clean Person and Dirty Person during a sampling event is crucial. Your role is to support each other during the sampling, to make sure that protocols are followed and that both team members are operating safely.
- **Rational thinking:** Be calm, be confident. If you are acting as a sampler, you have demonstrated your ability to safely conduct this carcass sampling. Refer to the written procedure, if you need to. And remember, *if in doubt, disinfect.*
- **No obligation:** Participation in WCS' Minimal-Exposure Carcass Sampling programme is voluntary. During training the level of the trainee's competence, comfort and willingness to perform the sampling will be evaluated. If, in the sole judgment of the instructor(s), a trainee is judged not ready to conduct carcass sampling (e.g. is highly fearful, does not demonstrate adequate understanding of the principles or cannot adequately perform the necessary tasks) he/she may be eliminated as a sampler candidate until they are judged to be capable. Likewise, if at any time during, or on completion of training a trainee does not feel comfortable and would like to withdraw, they may do so. If a sampler does not feel comfortable responding to a request to sample a carcass, they may decline the request and another sampler will be sought for the task. If, at any time during a sampling event, a sampler does not feel comfortable continuing with the procedure, he/she may discontinue it. In that instance, the Sampling Team may move to the cleanup and doffing phase of the protocol. Except in extreme emergency (e.g. a life-threatening danger to the sampler, such as aggression or dangerous animal encounter), any and all contaminated materials must be properly disinfected, processed and disposed of, according to the protocol.

Definition of Terms

Biological sample – Any material taken from an organism for analysis, including blood, fluids, tissue, feces, etc.

Biohazard material – Any materials potentially contaminated with dangerous pathogens.

Pathogen – Microorganisms, such as viruses, bacteria, fungi and parasites that can cause disease.

Sampler – A qualified individual, properly trained in the Minimal-Exposure Carcass Sampling Protocol, who will act as either the Clean Person or Dirty Person during a Carcass Sampling Event.

Clean Person – The Clean Person will assist the Dirty Person, closely observing the Dirty Person for proper procedure, providing disinfection as needed or requested by the Dirty Person. The Clean Person remains on the Clean Side at all times and never touches the Dirty Person, the Working Surface or the sampling materials. Once an object touches the Working Surface the Clean Person must NOT touch it again. If the Dirty Person needs material (e.g. another plastic bag), the Clean Person carefully *drops the item onto* the Working Surface.

***NOTE:** The spraying of disinfectant is done from a distance; the tip of pump sprayer should never be closer than 20 cm (about the length of your forearm) away from a potentially contaminated surface.*

Dirty Person – The Dirty Person will conduct the carcass sampling, collecting samples from the carcass, placing them in the appropriate sample containers, closing the containers and triple-packaging them. The Dirty Person remains on the carcass side of the Red Line (Dirty Zone) at all times, from the placement of the Working Surface to the removal of the PPE.

PPE – Personal Protective Equipment; equipment designed to protect you from contact with pathogens. Includes waterproof Tyvek™ protective coveralls, N95 facemask, goggles and nitrile gloves.

Donning – Refers to putting on PPE. Donning is always performed with a “buddy” who observes and assists with proper and secure PPE placement.

Doffing – Refers to the process of removing PPE after use. Doffing of PPE is the stage that carries the highest risk of contamination, so is arguably the most critical part of the carcass sampling process. Doffing is always performed with a “buddy” who closely observes and advises, and provides disinfection at every stage of the doffing process to ensure proper and safe PPE removal.

Nitrile gloves – Nitrile gloves are thicker, more durable and have 4X the puncture and tear resistance of latex gloves. They are also hypoallergenic, so can be worn by individuals with an allergy to latex.

Cuff – The portion of the glove that covers the wrist.

Cleaning – Removing of organic material (e.g. dirty, feces, blood) from a surface using physical (e.g. scrubbing), chemical (e.g. soap, detergent) means.

Disinfection – Disinfection refers to killing of microbes on or in an object, such as skin, gloves or a tabletop. Organic material on a surface can impede the penetration of a disinfectant, so for disinfection to be effective, the surface to be disinfected should ideally be first cleaned of organic material.

Sampling Event – Refers to the entire procedure, from arrival at the carcass through to the proper disposal of medical waste.

Work Area – The general area including the carcass and up to about 10 m from the carcass where sampling will take place. It includes both the Dirty Zone and the Clean Zone. Only Samplers are allowed in the Work Area.

Clean Zone – The side of the Work Area furthest from the carcass.

Dirty Zone – The side of the Work Area where the carcass is located and which is considered potentially contaminated with pathogens.

Red Line – An arbitrary line, approximately 3-5 m from the carcass, and within the Work Area, marked with red plastic tape that separates the Dirty Zone from the Clean Zone.

Working Surface – The surface, formed by a plastic bag or plastic sheeting placed on the ground, on which sample processing (placement of samples into sample tubes and packaging of samples) takes place and on which sampling materials (plastic base, sample tubes, zip-closure plastic bags) are placed. The edge furthest from the carcass touches the Red Line, so the Working Surface is completely within the Dirty Zone of the Work Area.

Carcass sampling form – Printed form on which details of the carcass and sampling event are recorded for the laboratory.

Carcass sampling kit – Refers to the collection of all required materials needed to conduct one Sampling Event.

AVL buffer – A preservative liquid that inactivates (kills) viruses and other pathogens in biological samples.

Sample tube – Small plastic screw-top, leak-proof containers (2 mL vials) that will hold carcass sample swabs in AVL buffer.

Sample bag – Leak-proof zip-closure plastic bag for securely packing the sample tubes for safe transport.

Sample Transport Container (or Bio-bottle) – A sturdy screw-top, hard plastic jar that allows safe transport of samples to the laboratory.

Biohazard bag – Large, heavy-duty plastic bags for containing and transporting all potentially contaminated disposable waste, including Tyvek™ coveralls, boots, gloves and masks.

Pump sprayer – A 3-5 L plastic jug that uses compressed air to spray the 5% sodium hypochlorite disinfectant. These are often used for spraying weed killer or plant fertilizer and will be used to spray and disinfect during the procedure.

Disinfection bucket – A 7-15 L plastic bucket in which you will put 5% sodium hypochlorite disinfectant and which is used to disinfect the reusable items and the Sample Transport Container.

Plastic base – A small polyethylene sample tube rack that will hold the sample tubes steady during the sampling process.

Triple packaging – A redundant safety measure for safely packing biological samples whereby potentially infectious materials (e.g. sample tubes or used PPE) are packaged in three successive layers, with disinfection used at each layer (materials within a bag, within a bag, within a bag).

Carcass Sampling Protocol

Establishing the Work Area and Red Line

NOTE: You will need 10 L of water for the sampling event. Since water is often not available at a carcass site, it is wise to obtain water in advance and carry it in.

Assessing the Situation:

- On arrival in proximity to the carcass and at a safe distance (minimum 20-30 m from the carcass) stop, get your bearings and prepare your materials. Any non-Sampler persons (e.g. the hunter who reported the carcass, porter(s)) should remain at this distance from the carcass:
- Decide who will take the “Clean Person” and “Dirty Person” roles;
- Take a GPS recording, preferably in decimal degrees;
- Remind yourself of the short protocol before beginning.

The Clean Person prepares disinfectant while the Dirty Person completes the forms:

- Put ~5 L of water into the bucket and add 1 sodium hypochlorite (‘bleach’) tablet (or ½ L liquid bleach). The chlorine bleach tablets will dissolve while you are preparing the site and materials. This will produce a 5% bleach solution which will be used to disinfect materials during the sampling procedure;
- Put ~3 L of water into the pump sprayer, add 1 sodium hypochlorite (‘bleach’) tablet (or ½ L liquid bleach; which will be stronger than a 5% bleach solution). The chlorine bleach tablets will dissolve while you are preparing the Working Surface and materials. This will be used for spraying and disinfecting all the materials, PPE during doffing, and the waste bags after, the sampling procedure;
- Keep the remaining fresh water (~2 L) to rinse goggles and other reusable items after sampling and disinfection;
- Make sure the disinfectant tablets in the pump sprayer are well dissolved. Securely close the pump sprayer and pressurize it using the pump handle.

Completing the Carcass ID Card and Notebook Page (Dirty Person):

The Carcass ID Card and Notebook Page are intended to provide those receiving the samples as much information as possible without having to open the triple packaging on the samples. The card also provides additional detail that would not fit on the sample tube label. The Sample Notebook will also allow the collectors, WCS staff and other researchers to quickly identify the contents of a transport container, the sampling team, the sampling location and other important information.

- Take a minute to complete the carcass sampling forms in the notebook and the card packed in the sample collection kit.
 - Provide as much of the information as you can, including notes on carcass species, age of the carcass, sex, etc.. If more information is gathered during the actual sampling it can be added later to the notebook page. The carcass ID card **MUST** accompany the Sample Transport Container – it will be bagged and placed into the Sample Transport Container along with the samples.
- Place the sample ID sticker on the Sample Transport Container;

- Place the completed Carcass ID Card in its plastic zip-closure bag and securely seal the bag.

The Dirty Person establishes the RED LINE:

Before setting up your materials, it is important to get your bearings and establish the “Red Line”, separating the Dirty (carcass, Working Surface) Side from the Clean (non-carcass) Side of your Work Area. ONLY the Dirty Person may pass beyond the Red Line and ONLY wearing full PPE.

- The Dirty Person opens the carcass sampling kit and puts on a pair of nitrile gloves and a N95 mask over their nose and mouth and pinches the metal bar tightly over their nose;
- The Dirty Person carefully approaches the carcass with the Red Line biohazard tape, clearing the vegetation with a machete as necessary, remaining **minimum of 3 m** from the carcass, any parts or liquids associated with the carcass.
- Determine the best direction from which to approach the carcass for sampling (*Note: It may be easiest to approach and sample from another direction*);
- Identify a site approximately 3 m from the carcass (including parts and/or any associated liquids) where you will establish the Red Line and your Working Area. Do not enter the work area or approach the carcass any closer than the work area as it will be on the Dirty Side. The Work Area will be where you set up your sampling materials. The area should be close enough to allow easy movement between the Work Surface and carcass once you are fully suited-up. The area should be flat and dry, with minimal undergrowth;
- Using the red biohazard tape, a stick or the machete, trace a straight line on ground to delineate the “Red Line”;
- As the Dirty Person returns, and before putting on the PPE the Clean Person will spray the Dirty Person’s boots and hands (with gloves) with the freshly made bleach solution.

Donning (putting on) PPE

Donning of PPE must be done with careful attention to detail. Each Sampler should assist the other, making sure that PPE is correctly and securely placed, zippers fully zipped, skin adequately covered, etc. Don the PPE one by one, assisting each other to ensure the correct order and placement of the PPE. Check each others’ PPE at the end. Once you begin putting on the PPE you will begin getting hot and sweaty.

Dirty Person

- To facilitate the safe removal of goggles at the doffing stage, add a tape “pull tab” to the elastic strap of the goggles. Place a 15 cm piece of tape around the center of the elastic strap of the goggles, folding it back onto itself to form an extended “pull tab”;
- Put on the Tyvek™ coveralls:
 - If wearing standard hard soled, closed toe shoes - step directly into the Tyvek suit wearing your shoes (Note that only closed-toed shoes or boots should be worn; NEVER bare feet or sandals);
 - If wearing very large Wellington boots – one foot at a time, step out of the boot, put into the foot of the Tyvek suit and step back into the boot.
- Put your arms in the sleeves, close the front zipper;
- The Dirty Person should already be wearing the first pair of gloves. Ensure the cuffs of the gloves are tucked **under** the sleeve of the coveralls;
- Put on the *second* pair of gloves over the first pair, pulling the cuff of the glove **over** the sleeve of the coveralls;

- Tape the second pair of gloves to the sleeve of the coveralls to keep it from gapping when you move your arms (**NOTE:** This is where teamwork is very important. Have your sampling partner place the tape while you hold your glove and sleeve in place.)
- Put on the *third* pair of gloves over the second pair. This pair will not be taped;
- The Dirty Person should already be wearing their N95 mask;
- Pull up the attached hood (this will cover your head and goes *over* the straps of the respiratory N95 mask). All hair and as much skin as possible must be covered by the hood;
- Put on the safety eye goggles over the hood. Make sure that the eye goggles are securely fitted to your face and covering as much of your skin as possible. Make sure that the hood is pulled far enough forward and underneath the top of the goggles in order to completely cover the forehead;
- Place the boot covers over the bottom of the boots.

Clean Person

- To facilitate the safe removal of goggles at the doffing stage, add a tape “pull tab” to the elastic strap of the goggles. Place a 15 cm piece of tape around the center of the elastic strap of the goggles, folding it back onto itself to form an extended “pull tab”;
- Put on the Tyvek™ coveralls:
 - If wearing standard hard soled, closed toe shoes - step directly into the Tyvek suit wearing your shoes (Note that only closed-toed shoes or boots should be worn; NEVER bare feet or sandals);
 - If wearing very large Wellington boots – one foot at a time, step out of the boot, put into the foot of the Tyvek suit and step back into the boot.
- Put your arms in the sleeves, close the front zipper;
- Put on the *first* pair of gloves of nitrile gloves, tucking the cuff ***under*** the sleeve of the coveralls;
- Put on the *second* pair of gloves over the first pair, pulling the cuff of the glove ***over*** the sleeve of the coveralls;
- Tape the second pair of gloves to the sleeve of the coveralls to keep it from gapping when you move your arms (**NOTE:** This is where teamwork is very important. Have your sampling partner place the tape while you hold your glove and sleeve in place.)
- Put on the *third* pair of gloves over the second pair. This pair will not be taped;
- Put on the N95 mask, covering nose and mouth, and pinch the metal strip over the nose to ensure a secure and comfortable fit;
- Pull up the attached hood (this will cover your head and goes *over* the straps of the respiratory N95 mask). All hair and as much skin as possible must be covered by the hood;
- Put on the safety eye goggles over the hood. Make sure that the eye goggles are securely fitted to your face. This may require pulling the sides of the hood back, so that the hood material rests on the outside of the goggles. Make sure that the hood is pulled far enough forward to touch the top of the goggles and completely cover the forehead.

Check each sampling partner’s PPE, making sure that zippers are fully and properly closed, that the second pair of gloves are securely taped to sleeves, and that mask and eye goggles are correctly placed. Assist your partner with any adjustments that need to be made.

Preparing the Work Area

Dirty Person organizes the Dirty Side:

Without crossing the Red Line yet, establish and organize the work area *from the Clean Side*.

- Spread one of the biohazard bags on the ground on the Dirty Side, touching the Red Line. This will act as your Working Surface;
- Organize the sampling materials on the Working Surface (sample tubes containing AVL, plastic base, swabs, sample bags, Sample Transport Container, a biohazard waste bag a spare pair of gloves, disinfectant bucket and the filled sample ID card in its plastic bag). Arrange the plastic bags for the sample tubes in the order in which they will be used. Opening the zip closure on each bag will make packaging the sample bottle later easier and will save time;
- The Dirty Person now steps across to the Dirty Side of the Work Area. FROM THIS POINT ONWARD, THE DIRTY PERSON REMAINS IN THE DIRTY ZONE AND THE CLEAN PERSON REMAINS IN THE CLEAN ZONE;
- Fully open one biohazard bag and place it on the ground on the Dirty Side, next to the working surface and the Red Line; roll the edges outwards and downwards, using your hands to open the center as much as possible. The prepared bag should resemble a giant bowl into which you can easily place contaminated items;
- In order to allow safe, unencumbered access to the carcass, you may need to clear some vegetation. Using a machete or secateurs (plant nippers), clear enough foliage/undergrowth to allow yourself to work comfortably.

Clean Person organizes the Clean Side:

- While the Dirty Person is organising the Dirty Side, the Clean Person arranges the pump sprayer, PPE, biohazard waste bag and a large biohazard transport bag and spare materials on the Clean Side. The pump sprayer, biohazard waste and transport bags, spare mask and gloves will remain in the Clean Zone at all times;
- Make sure disinfectant tablets in the disinfectant bucket are well dissolved;
- Without crossing the Red Line, place the disinfectant bucket on the Dirty Side, next to the Working Surface and near the Red Line. Open the Sample Transport Container and set it aside on the Working Surface;
- Fully open one biohazard bag and place it on the ground on the Clean Side, next to the working surface and the Red Line; roll the edges outwards and downwards, using your hands to open the center as much as possible. The prepared bag should resemble a giant bowl into which you can easily place contaminated items.
- Fully open a *second* biohazard bag (slightly larger) and place it on the ground on the Clean Side, a couple of meters from the Red Line, this is the Biohazard Transport Bag; roll the edges outwards and downwards, using your hands to open the center as much as possible. Into this bag will be placed the Dirty Biohazard and Clean Biohazard bags for transport later in the protocol.

Using insecticide

If the situation calls for insecticide use the following steps:

- Place insecticide sprayer on the Dirty Side;
- After the Dirty person cuts any additional brush around the carcass use the sprayer until it is finished to kill any insects on or around the carcass;
- Disinfect the sprayer completely and place in the Dirty Biohazard bag;
- Proceed with the protocol

Using a camera

Photographs of the carcass can be very useful providing further information of the carcass. Use the following steps:

- If you have a waterproof case you may use the camera on the Dirty Side; disinfect the case as you would the machete;
- If you do not have a waterproof case, the camera must not cross the Red Line; the Clean Person will take photographs immediately after the Dirty Person has cleared any brush from near the carcass. Place the camera with the GPS for transport.

Sampling Carcass (Dirty Person)

- If needed, use the machete or secateurs to cut additional underbrush to facilitate safe access to the carcass. Place the machete (or secateurs) blade-first into the disinfectant bucket. The Clean Person will spray any remaining parts of the machete not yet covered in the disinfectant. Once disinfected, the clean person can remove machete and place on the Clean Side (this will help samplers avoid sharp contact from the machete while reaching in and out);
- Dirty Person removes the screw tops from the 4 sample vials and place them on the vial holder on the Working Surface;
- Remove 4 swabs from their packaging. Touching only the plastic shafts, proceed to the carcass. Do not rush. Take your time;
- Visually examine the carcass for any signs that may indicate the cause of death (e.g. wounds, broken bones, presence of a wire snare, etc.). Make a mental note or communicate the information to the Clean Person. You can record this information later on the carcass sampling notebook;
- Holding the swabs by the plastic shaft, with glove clearly away from the cotton tips, swab the carcass and/or carcass fluids.
 - Hold all the swabs together in a bundle to collect the sample onto all the swabs simultaneously;

- Swabs may be dipped in liquid, wiped on a surface or stuck into soft tissue;
- Areas protected from sunlight are preferred. Make sure the swab gets as much liquid or material on it as possible;
- The best sample for diagnostic testing is bloody or moist tissue. Mouth and nose are best, but these are often the first to decay, so may not be easily sampled.
- Return to the work surface being careful not to let the swabs touch anything, especially you;
- One swab at a time, place each swab, tip first, into a sample tube;
- Stabilising the sample tube with one hand, carefully bend the plastic shaft of the swab to break it off. Place the plastic shaft directly into the Dirty Side waste biohazard bag. Repeat for the remaining swabs;
- Replace the screw tops onto the sample tubes and *close them tightly*;

Place the tube holder and the sample tubes into the disinfectant bucket for disinfection. Submerge the tubes in the disinfectant bucket and leave for a minimum of 10 minutes.

Triple Packaging (“Triple Emballage”) of Samples

All potentially infectious biological samples must be secured in triple packaging. The Dirty Person performs this while the Clean Person sprays disinfectant.

- After 10 minutes of disinfection place the sample tubes in the *first* plastic sample bag;
- Disinfect the inside and outside of the first sample bag by spraying or dipping the open bag in the disinfectant bucket, then pour out any excess disinfectant;
- Close the sample bag zip closure, removing air from the bag as you do so, and place it in the *second* sample bag;
- Disinfect the inside and outside of the second sample bag, as before, then pour out any excess disinfectant;
- Close the sample bag zip closure, removing air from the plastic bag as you do so, and place it in the *third* (labeled) sample bag; as before, then pour out any excess disinfectant;
- Close the sample bag zip closure, removing air from the bag as you do so, then place it in the Sample Transport Container;
- Disinfect the outside of the carcass ID card (in a sealed bag) and place it in the Sample Transport Container;
- Close the lid of the Sample Transport Container, then place the complete closed Sample Transport Container in the disinfectant bucket and leave for a minimum of 10 minutes.
- The Clean Person sprays the working surface with disinfectant;
- Touching only the underside of the Working Surface, and beginning at the corners, the Dirty Person slowly folds the edges of the Working Surface into the center to form a package small enough to easily fit into the biohazard bag. The Clean Person continues to spray with disinfectant while the Dirty Person carefully places the folded Working Surface into the Dirty Side biohazard bag;
- Spray around the opening of the Dirty Side biohazard bag.

Changing gloves

If at any time a Dirty Person's **glove** becomes **torn** or **punctured**, **stop and change it:**

- Thoroughly disinfect your gloves;
- You should have a spare pair of gloves on the Working Surface;
- Touching only the cuff of the damaged glove, carefully peel it off, away from the body and the Clean Person;
- Drop the used glove into the Dirty Side biohazard bag;
- Put on the new glove and resume work.

Doffing (removing) PPE

The greatest risk of infection accompanies the doffing of PPE, making this step perhaps the most critical step of the process. At this stage, Samplers will behave as if the outside of the protective materials are potentially contaminated, meaning that at no time does the Sampler (or his/her overseeing "buddy" allow a "clean" surface to touch an external "dirty" surface, except as specified. The **Dirty Person** removes his/her PPE first while the Clean Person disinfects by spraying at every stage of the process, observes for any breach of protocol and supports by instructing the correct order, if needed:

The Dirty Person removes his/her PPE:

- **DISINFECT** – ask the Clean Person to spray your gloves and entire front of your Tyvek coveralls and your booties with the bleach solution disinfectant; turn around and do the same on the back (*including the bottoms of the boot covers!*);
- Remove the boot covers and step into the now empty area where the working surface had been;
- Place the boot covers into the Dirty Side biohazard bag. Disinfect the exterior pair of gloves;
- Remove the *third* (exterior) pair of gloves – touching only the exterior surface of the gloves, avoiding contact with the second pair of gloves beneath – and carefully drop them into the biohazard bag. Disinfect the now exposed second pair of gloves;
- Still wearing the second "clean" pair of gloves, remove your eye goggles, touching only the "pull tab" on the elastic strap and not the plastic goggle themselves (reach to the back of the hood, grasp elastic strap between finger and thumb and carefully remove the goggles upward, away from the face), and drop the goggles into the disinfectant bucket; disinfect gloves and pull down the hood (from the top); disinfect gloves and slowly and carefully, fully unzip the coveralls. Disinfect the second pair of gloves;
- Remove the strip of tape from the second pair of gloves, first one, then the other, carefully dropping each strip of tape into the "dirty" biohazard bag;
- Disinfect your gloves again;
- With the clean second glove, touching only the *inside* of the Tyvek™ coveralls, begin removing the coveralls:
 - Remove arms, turning the sleeves in-side-out as you do so;
 - Peel the coveralls away from the body and legs, touching only the *interior* to roll them down and to the outside;

- If wearing Wellingtons – remove your foot from the boot, pull your foot out of the suit allowing the leg and foot of the suit to turn inside out. Without letting your foot touch the ground replace it into your Wellington. Repeat with the other foot.
- The clean person will spray the boots as the Dirty Person steps to the Clean Side
- If wearing other hard soled, closed toe shoes - pull your foot out of the suit allowing the leg and foot of the suit turn inside out. As you remove your shoed foot from the Tyvek, **STEP ACROSS THE RED LINE TO THE CLEAN SIDE (DO NOT PLACE STEP WITH THE UNPROTECTED SHOE ON THE DIRTY SIDE)**;
- Touching only the interior of the Tyvek coveralls, carefully fold them into a small package that will easily fit inside the biohazard bag. Clean Person continues to spray the Tyvek as the Dirty Person folds the Tyvek coveralls;
- Place the folded coveralls in the Dirty Side biohazard bag;
- Disinfect your gloves;
- Touching only the *exterior* portion of the cuff, remove the *second* pair of gloves and, *without stepping into the Dirty Area*, carefully drop them into the Dirty Side biohazard bag;
- Disinfect your gloves;
- Grasping the *front* of the mask between thumb and forefinger, remove your mask. *Without stepping into the Dirty Area*, carefully drop your mask into the Dirty Side biohazard bag;
- Disinfect your gloves;
- Touching only the exterior surface, carefully remove your remaining pair of gloves. *Without stepping into the Dirty Area*, carefully drop your gloves into the Dirty Side biohazard bag;
- Step away from the Red Line, and don a new face mask and a new pair of gloves.

While the Dirty Person dons a new mask and gloves, the **Clean Person** disposes of the Dirty Side biohazard bag and disinfects the disinfectant bucket, without stepping into the Dirty area:

- Thoroughly spray the handle and outside of the bucket of disinfectant;
- Thoroughly spray the Dirty Side biohazard bag with disinfectant, both the opening and the exterior of the bag;
- Without stepping into the Dirty Area, reach across the Red Line and retrieve the Dirty Side Biohazard Bag. Avoiding touching the opening and grasping low on the bag to minimize trapping air in the bag, carefully close the bag using a zip tie and roll into a package small enough to easily fit into the Clean biohazard waste bag. (*NOTE: Try to begin as low as possible on the bag, so as to minimize trapping air in the bag. DO NOT squeeze the bag to expel air.*)
- Without stepping into the Dirty Area, the Dirty Person reaches across the Red Line and brings the disinfectant bucket to the Clean Side;
- By this time, the Dirty Person should have donned his/her new mask and gloves. If he/she has not, the Clean Person will wait for the Dirty Person to do so. Once the Dirty Person has donned new mask and gloves, he/she takes the disinfectant pump sprayer;
- The Dirty Person disinfects the Red Line while the Clean Person places it in the Clean Biohazard bag.

The **Clean Person** now removes his/her PPE while the **Dirty Person** disinfects, observes and instructs as necessary.

- *DISINFECT* – ask the Clean Person to spray your gloves and the entire front of your Tyvek coveralls and booties with disinfectant; turn around and do the same on the back (*including the bottoms of the boot covers!*);
- Remove the boot covers and place them into the Clean biohazard bag. Disinfect the exterior pair of gloves;
- Remove the *third* (exterior) pair of gloves – touching only the exterior surface of the gloves, avoiding contact with the second pair of gloves beneath – and carefully drop them into the “clean” biohazard bag. Disinfect the now exposed second pair of gloves;
- Still wearing the second pair of gloves, remove your eye goggles, touching only the “pull tab” on the elastic strap and not the plastic goggle themselves (reach to the back of the hood, grasp elastic strap between finger and thumb and carefully remove the goggles upward, away from the face), and drop the goggles into the disinfectant bucket; pull down the hood (from the top); slowly and carefully, fully unzip the coveralls. Disinfect the second pair of gloves;
- Remove the strip of tape from the second pair of gloves, first one, then the other, carefully dropping each strip of tape into the “clean” biohazard bag;
- Disinfect your gloves again;
- With the clean second glove, touching only the *inside* of the Tyvek™ coveralls, begin removing the coveralls:
 - Remove arms, turning the sleeves in-side-out as you do so;
 - Peel the coveralls away from the body and legs, touching only the *interior* to roll them down and to the outside;
 - Touching only the interior of the coveralls, pull them off of one foot, turning the boot in-side-out;
 - Repeat for the other foot;
 - Touching only the interior of the Tyvek coveralls, carefully fold them into a small package that will easily fit inside the biohazard bag.
- Disinfect the folded coveralls and place them in the “clean” biohazard bag;
- Disinfect your gloves;
- Touching only the *exterior* portion of the cuff, remove the *second* pair of gloves and carefully drop them into the “clean” biohazard bag;
- Disinfect your gloves;
- Grasping the front of the mask between thumb and forefinger, remove your mask and carefully drop your mask into the “clean” biohazard bag;
- Disinfect your gloves;
- Touching only the exterior surface, carefully remove your remaining pair of gloves and carefully drop your gloves into the “clean” biohazard bag. The Clean Person will not handle any material from this point onwards, and the Dirty Person will finalise the disinfection procedures;
- The Dirty Person sprays the “clean” biohazard bag with disinfectant, both the opening and the exterior of the bag;
- The Dirty Person will, without touching the opening and grasping low on the bag to minimize trapping air in the bag, carefully close the bag using a zip tie. Roll into a package small enough to easily fit into the Biohazard Transport Bag and spray down prior to placing into the Biohazard Transport Bag. (*NOTE: Try to begin as low as possible on the bag, so as to minimize trapping air in the bag. DO NOT squeeze the bag to expel air.*);

At the end, all materials brought to the Work Area should be either disinfected or disposed of by burning.

- Place all non-reusable items in the transport bag (empty PPE bags, unused spare gloves and zip ties)
- Ensure all items not to be burned are disinfected in the sodium hypochlorite bucket.

Transport of Biological Samples:

- Once sample items have been in the disinfectant for 10 minutes, they are considered fully decontaminated and can be retrieved.

Rinsing Reusable Items:

- Once reusable items have been in the disinfectant for 10 minutes, they are considered fully decontaminated and can be retrieved. Use the remaining fresh water in the plastic jerry can to rinse the reusable items.

NOTE: *Chlorine bleach is highly corrosive to metal, so should be rinsed from the reusable items.*

- Remove the goggles, plastic base and rinse them;
- Thoroughly rinse the machete and/or secateurs;
- Empty the remaining disinfectant from the bucket on the Clean Side and rinse it out;
- Place the goggles, tube holder, secateurs and Sample Transport Container back into the bucket for transport.

Dirty Person will carefully close the Biohazard Transport Bag:

- Spray the outside of the biohazard bag with disinfectant, especially around the top/opening;
- Also disinfect the outside and the handle of the pump spray;
- Remove your masks and then the pair of gloves and place them in the Biohazard Transport Bag;
- Touching only the exterior of the biohazard bag, slowly twist the neck of the bag repeatedly, gently pressing the contents to the bottom. (*NOTE: Try to begin as low as possible on the bag, so as to minimize trapping air in the bag. DO NOT squeeze the bag to expel air.*);
- Use a zip tie to secure closure of the twisted “neck” of the bag;
- Spray the outside of the Biohazard Transport Bag with disinfectant around the top/opening.
- The biohazard bag is now safe to handle and transport (see Disposal of Biohazard Materials below).

Disposal of Biohazard Materials

All biohazard bag and remaining PPE are to be disposed of by burning to ensure that all potential pathogens are destroyed and do not pose a risk of infection to people or animals. Burning should be conducted as soon as safely possible after completing the carcass sampling, away from human habitation. You may need fuel to encourage the fire:

- Carefully place the biohazard bag onto the fire or set fire to the biohazard bag;
- You may need to carefully turn portions of the fire with a stick periodically to ensure that all materials are *fully burned*;
- Once materials are *fully burned*, fully extinguish the fire before leaving the area.

NOTE: *If burning cannot be safely done on site, the biohazard bag may be transported to another site (e.g. base camp etc.) for burning.*

NOTE: *The Transport Container is to be opened only by trained personnel under controlled laboratory conditions. Under no circumstances is the Sample Transport Container to be opened during transport. Samples must remain at all times in the possession of the sampler or a responsible WCS staff person who is well informed of the transport purpose. Transport of samples to the laboratory should be done discretely, but as expediently as is feasible. Do not allow a non-WCS person to transport samples. Protect the samples from excessive heat and sunlight at all times by keeping the Sample Transport Container in a shaded, cool place. Do not expose the container to excessive heat, such as in a closed, parked vehicle or the hot floor of a car during transport. On arrival at the laboratory, the samples should be delivered to laboratory personnel. Under no circumstances are samples to be left unattended or given to a non-WCS person unless specifically instructed to do so by WHP.*

Appendix I

Sample Labeling

Providing detailed information for any biological samples is critical to the usefulness of the samples. A sample that tests positive for a given pathogen is useless if we don't know where it came from, when or what species it came from. The following information should be included with ALL samples:

- **I.D.** – the individual animal's name (e.g. if part of a known group) or other identification, if known;
- **Species** – identify the species to appropriate level (e.g. *gorilla*, *G. g. gorilla*, etc.);
- **Sex** – Based on the size and external characteristics of the animal, determine the sex as best you can (**M** = male; **F** = female; **Unk** = unknown);
- **Age** – Based on the size and external characteristics of the animal, determine the age class as best you can: **A** (Adult, includes silverback male), **SA** (Sub-Adult; includes black back), **J** (Juvenile), **N** (neonate);
- **Zone** – the zone where the carcass is located (e.g. FMU Ngombé, Mbeli Bai, OKNP, etc.);
- **GPS** coordinates – latitude & longitude. Be clear about format (use decimal degrees NOT hours, minutes, seconds);
- **Preservative** – The preservative – AVL – is indicated on the sample bottle label.
- **Date** – date of sampling, in the format indicated;
- **Sampler** – Initials of the two samplers.

C1

Carcass Sample AVL

Fig 1. Sample bottle label. The sample bottle label (sticker) is very small, so only contains a minimum of information: Species, date and samplers' initials.

C1

Carcass Sample

Esp: gorilla Animal ID:

N/A

Sex: M F Unk Age: A SA J N

GPS: N 1.04253 E 16.00892

Échant: swab in AVL

Prélèv: AO/DM

Date: 15 / 11 / 14 Zone: GLG

jj mm aa

Notes: _____

Fig 2. Sample ID Card and notebook page. The Sample number – C1 and sample description are printed at the top. The Species, here a gorilla, has been indicated. If the individual animal was identified, enter the ID here, if not indicate with N/A (not applicable). Sex and age should be indicated following the guidance above. The GPS coordinates are entered in decimal degrees. The type of sample is indicated; in this case swab. The Samplers, AO and DM, are indicated with initials. The Date is indicated in the appropriate format. The Zone has been entered; in this case GLG for Goulougo Triangle. The preservation medium, AVL, is indicated in print on the card. Any further observations can be recorded in the notes section. The Sample ID Card will go inside the Sample Transport Container with the Samples. The Notebook page will go into the collection team's notebook.

Appendix II

Materials / Equipment List

The following items are contained in the pre-packaged 2-PP carcass sampling kit:

2 PPE bags that contain:

- 1 Tyvek™ coverall with attached hood
- 4 Pairs of long gauntlet nitrile gloves
- 1 N95 respirator mask
- 1 Pair of boot covers

1 Sample collection bag that contains:

- 4 2mL Sarsteadt® red capped sample vials containing 1 mL AVL buffer each
- 4 Polyester-tipped swabs
- 3 Sample bags, labeled
- 1 Carcass ID card
- 1 Plastic bag for carcass sampling card
- 1 Notebook page form
- 1 Label for the Transport Container
- 4 Biohazard bags
- 1 3m plastic hazard ribbon to establish the Red Line
- 4 Zip ties
- 4 Bleach tablets
- 2 Pairs long gauntlet nitrile gloves
- 1 N95 respirator

The following items accompany the pre-packaged 2-PP carcass sampling kit:

- 1 5L pump sprayer* (or 3L pump sprayer) * reusable item
- 1 5L plastic bucket*
- 1 Roll of duck tape*
- 1 10L plastic jerry can, for water*

The following items are not included, but required:

- 2 Goggles*
- 1 Plastic base*
- 1 Transport container*
- 1 Machete or secateur*
- 1 10 Liters of water*
- 1 GPS device*
- 1 Pen or pencil*
- 1 Watch*
- 1 Hand sanitizer/soap for clean-up after sampling
- 1 Insecticide sprayer (disposable)

If burning on site, bring necessary fuel to complete the disposal of biohazard materials.