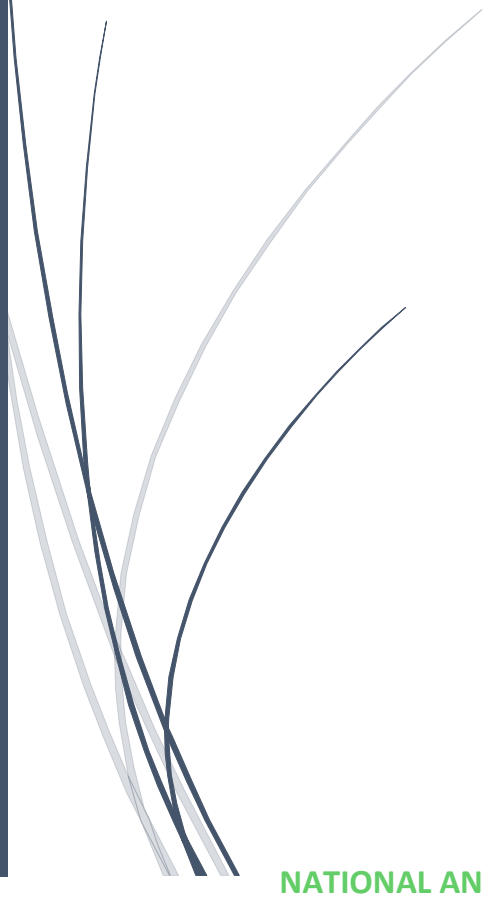
LABORATORY SPECIMEN SUBMISSION GUIDE



NATIONAL ANIMAL HEALTH DIAGNOSTIC AND INVESTIGATION CENTER

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Food and Agriculture Organization of the United Nations

MINISTRY OF AGRICULTURE

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PREAMBLE

The National Animal Health Diagnostic and Investigation Center (NAHDIC) is the referral and reference veterinary laboratory of Ethiopia. It is the center of excellence for animal disease surveillance, investigation, diagnosis and research. It was established in 1995 as a national disease investigation center. The center is found in Sebeta, which is located at 27kms from the capital, Addis Ababa. After two years of being a central disease investigation laboratory, it was mandated to handle both animal health research and diagnosis and organized under the then Ethiopian Institute of Agricultural Research (EIAR),and was named as National Animal Health Research Center (NAHRC). Since 2007 the center has been mandated to focus mainly on diagnostic and investigative services along with additional duties and responsibilities. Most of its activities are related to supporting the animal and animal products export market of the country, improvement of the livelihood of the small-scale farmers and pastoralists, and advisory servicesto investors involved in animal farming.It is recognized that the value of the laboratory test results is directly dependent on the submission of quality and appropriatesamples, including volume,as well as the manner in which samples are handled, preserved and transported**.**Samples may be taken from individual animals, animal populations, or environment for a variety of purposes such as disease diagnosis, disease surveillance, health certification, and monitoring of treatment and/or vaccination responses. Hence,to provide quality and credible diagnostic services, NAHDIC requires its customer to follow standard sampling and submission guide during collection, storage, packing and transportation of samples. Hence, it is worthy to provide clients in general and animal health workers in particular withaguideline that indicates the recommended specimen (s)for various cases, preservatives to be used, and specimen handling procedures.

1. GENERAL INFORMATION ON SAMPLING AND SUBMISSION REQUIRMENTS

1.1 Sample Submission requirements to the NAHDIC

- NAHDIC accept and examine samples from all livestock including cattle, camels, sheep, goat, equines, poultry as well as wildanimals,pet animals, birds, fish and bees;
- A clearly dated sample submission form (NAHDIC-FRM-GEN-006),to be signed by submitter, should accompany sample or set of samples(Annex-1);
- Ensure that the submission form is fully completed and contains all relevantdetails. If additional detail (s) is required, it is possible to use separate memo sheet;
- Samples that do notcomplywith this guideline may not produce required result and hence may be subject to rejection;
- When live animals are intended for submission, prior consultation with NAHDIC'spertinent section or staff is required (Annex-2).

1.2 Labeling of Samples

Sample containershould contain at least animal ID, date of sampling and type of sample.

All basic additional information relevant to the sample should be completed in NAHDIC-FRM-GEN-006 form (Annex-1)

Every single sample should be labeled or marked with the following basic information (Annex-1).

- Type of sample(e.g.serum, whole blood, liver, brain, urine, etc.)
- Whether preservativeis used or notand type of preservative used(if any)
- Identification of animals (e.g. tag, name, sex, colors of hair coat, etc.) if samples are from more than one animal.
- Date and place of collection.
- The names of the owner, if samples from different cases are packed together in the same parcel.

1.3 Handling and Transport of Samples

- Hand delivered sample are more preferred;
- The parcels should be securely packed in order to withstand rough handling and protect shipper and the environment;
- Great care should be taken to ensure that the containers with sample (s) do not leak! (a sticky tape may be used);
- Use cotton wool, newspaper or other absorbent materials to soak up any leakage that, despite all the precautions, might occur;
- Use cooler boxesfor shipping refrigerated samples;
- For sample storage and special transport requirements for specific tests, refer to section 5.1.

2. PRESERVATIVES AND FIXATIVESTO BE USED FORSAMPLES

Some laboratory tests are not compatible with specific blood anticoagulants and tissue preservatives, such as heparin, formalin, dry ice (exposure of the test sample to elevated levels of CO2), or even freezing. While it is critical to collect specimens as aseptically as possible, equal care must be taken to avoid contamination with detergents and antiseptic treatments used to clean the collection site on the animal, as these agents may interfere with the laboratory test procedures. Procedures requiring tissue culture of pathogens as well as many molecular-based tests can be negatively affected by chemicals or detergents commonly used in the manufactureor preparation of collection tools (e.g. chemicals used in manufacture of some types of swabs and detergents used in cleaning glassware).

2.1 Refrigeration ”on ice’’

In general, if there is a delay between sample collection and submission to the laboratory, samples should be refrigerated.

- Refrigeration should be used to preserve any sample that is subject to rapid biological deterioration, e.g., tissue samples for bacteriological or virological examination, small body samples for post mortem examination, blood, feces or urine samples, etc.;
- During transportation use ice in the form of cubes or blocks or use camping refrigerant packs;
- The ice should be put in double tied-leak-proof plastic bags to avoid wetting of the samples;
- The ice bags or refrigerant packs must be placed next to the sample bag(s) in an insulated box or vacuum flask;
- The sample should be cooled down in a refrigerator before packing;
- After packing, the whole parcel should be kept in a refrigerator until immediately before dispatch.

2.2 10% Buffered formalin

- Used to preserve any tissues for histological/histopathological examinations;
- 10% buffered formalin is used at a rate of at least 10 volumes to 1 volume of tissue;
- The blocks of tissue should not be larger than 8 cubic centimeters (2cm of each side). Otherwise the preservation of the core of the tissue block will take too long;
- Samples intended for bacteriological or virological examinations should not be preserved with formalin**.**

2.3 50% Glycerol saline

- This preservative must only be used for submission of brain for rabies virus examination.

2.4 2.5% Boric acid

- Used to preserve tissues for bacteriologicalexamination;
- Samples preserved in this way do not require refrigeration during transport;

- Milk can be preserved for bacteriological examination by adding 4 volumes of 2.5% Boric acid to 1 volume of milk.

2.5 70% Alcohol

- Used for preservation of internal and external parasites;
- Use at least 2 volumes of 70% alcohol to 1 volume of sample.

2.6 Anticoagulants

Ethylene Diamine Tetra Acetic Acid (EDTA): used to collect whole blood forhematology and virology

Heparinised *Blood*: used for avian and wildlife hematology when submitted within 4 hours

- After collection of blood with anticoagulants mix thoroughly by gently inverting tubes several times;
- Blood samples submitted for other examination than routine should be submitted after prior consultation of NAHDIC.

2.7 Transport media

- Virus Transport Media (VTM) and Phosphate Buffered Saline (PBS)with antibiotics are used for samples suspected with viral diseases;
- Swabs in transport medium (semi-solid)are used to preserve fluid or semi-fluid samples for bacteriological or virological examination;
- Swabs can also be used for preservation of tissues of internal organs, if no ice or 2.5% boric acid is available;
- Dry swabs are not ideal for bacteriological examination;
- Samples for suspected cases of *Campylobacter* and *Trichomonas shinki* are taken in consultation with NAHDIC.

3. SAMPLING METHOD AND SAMPLE SIZE DETERMINATION

In herd or flock-based disease investigations, it is beneficial to collect sample according to a sound knowledge of the epidemiology and pathogenesis of the disease under investigation, surveillance or study. However, since it is rarely possible to sample and test all the animals (subjects) in a given population, sampling following a certain epidemiologically appropriate sampling procedure is necessary. Sampling is the process of selecting subgroup from a population.

Why do we use sampling?

- To reduce the time and cost of data collection;
- It is cheaper to collect data from a sample than from an entire population;

- Also, sample information can be collected and summarised more quickly since fewer elements are involved;
- By concentrating on fewer elements, more comprehensive data can be obtained from the sample than in a census.

3.1 Sampling type

Broadly speaking, there are two categories of sampling: probability (random) and non-probability (non-random) sampling. Probability or random sampling is often the best way to ensure that our sample is representative of the population. With probability or random sampling the aim is to give every animal in the population an equal chance of being selected for inclusion in the sample. However, in non-probability sampling, each member of the population does not have an equal chance of being included in the sample.

3.1.1Non-probability (non-random) sampling

Non-probability sampling is often called convenience sampling because It involves selection of the sample in such a way that is convenient for the surveyor but does not ensure that each member of the population has an equal chance of being included in the sample. There are three common types:

3.1.1.1 Purposive Sampling: selection of cases that have the required information with respect to the objectives of the study based on the elements possessing one or more attributes such as known exposure to a risk factor or a specific disease status.

3.1.1.2 Convenience Sampling: it is selection of cases as they become available to the researcher or investigator. Samples are chosen because it is easy to obtain (nearby herds, herds with good handling facilities, friendly farmers, etc.) and

3.1.1.3 Quota Sampling: this sampling method aims to include as many groups or quotas of the population based on some criteria

3.1.2 Probability (Random) sampling

In probability sampling, the usual practice is to select the sample in such a way that every animal in the target population has an equal chance of being included in the sample. When probability sampling is used, samples will, on average, be unbiased and it will be possible to calculate the precision of the estimate (i.e. put confidence limits around the estimate of the parameter). There are five types;

3.1.2.1. Simple random sampling: sample is chosen from the population directly in a one-stage process so that every animal in the population has an equal chance of being selected. Every animal in the sampling frame (list of all sampling units in the target population) is given an identification number. Then ‘n’ random numbers from ‘N’ possible numbers are chosen, using one of the methods (physically with dice, cards, numbers in a hat, with a random number table and using a computer to generate random numbers) where ‘N’ is the number of animals in the sampling frame and ‘n’ is the required sample size. Then, those animals with identification numbers corresponding to the selected random numbers are included in the sample

3.1.2.2 Systematic Sampling: sometimes it is not possible to come up with a sampling frame, but it is possible to order the animals/subjects in some manner. The classic example is cattle arriving in a line to a cattle dip. The sampling is done by taking every N/n^{th} animal where 'N' is the number in the population and 'n' is the required sample size. For example, if we had a population of 200 cows and we wanted a sample size of 20, we would take every10th (200/20) cattle to be included in the sample. The objective is the same as in simple random sampling; we want the probability of any one cattle's inclusion in the sample to be the same for all the cattle. To achieve this, we need to have a random starting number between 1 and 10. Suppose, by one of randomization methods the number 7 was selected. Then the seventh cow is selected and then the 17th, 27th, and so on.

3.1.2.3 Stratified sampling: stratified sampling is an approach to sampling where the sampling frame is divided into groups or strata based on characteristics likely to influence the property being estimated. Prior to sampling, the population is divided into mutually exclusive strata based on factors likely to affect the outcome (eg breed, sex, age, herd size, etc.), then a simple random or systematic random sample is taken within each stratum.

Stratification is useful because it allows separate estimates to be made for each strata; it is sometimes logistically easier to handle a number of smaller samples; it allows different sampling percentages to be used in different strata (ie n1/N1=5 % in stratum 1,n2/N2=2% in stratum 2); and the precision of the estimate may be improved if the animals are more alike for the property of interest (e.g. antibody prevalence) within the strata than between strata. Obviously, if stratified sampling is to be used then the characteristic which is used to stratify the population must be known for each unit (i.e. animal) in the sampling frame.

3.1.2.4 Cluster Sampling: often it is not possible to obtain a sampling frame like a list of individual cows in a district. More often, a list of natural or artificial groupings of the sampling units such as herds, pens, or litters is available. In this situation cluster sampling can be used. Cluster sampling starts with an initial random sample of groups. Then when the groups (farms, pens, villages) are selected, all the sampling units in each selected cluster (i.e. census of all animals in the herd) are measured or tested for the property of interest

3.1.2.5 Multistage Sampling: multistage sampling is very similar to cluster sampling except that random sampling takes place at each stage. In the cluster-sampling example above, every animal in the selected herds was measured or observed. However, in two stages sampling the first stage consists of the random selection of herds, as in cluster sampling, but then animals are randomly sampled from **within** each herd for the second stage. The larger group sampled in the first stage (i.e. herd) is called the primary unit and the sampling unit (i.e. animals) within the primary unit are called the secondary units**.**

3.2 Sample size determination

Choosing the correct sample size to ensure the statistical power to find the information can be a complicated endeavor for which one might need additional expert assistance. However, we can consider the calculations for sample sizes for some simple cases.

3.2.1. Sample Size for Estimation of a Proportion (π)

If L is the required precision, (+ or – error around estimate), p is the anticipated prevalence or proportion of attribute, Z is level of confidence at estimate of 95 %; Then the required sample size is calculated as follow:

$$n = \frac{Z^2(n-1)}{L^2}$$

3.2.2 Sampling to detect new (exotic) diseases

Sometimes, the question is not the level of disease but whether or not the disease is present. For example, if a producer is buying replacement animals, he/she may wish to be certain that the disease is unlikely to be present. In other circumstances, it may be useful to know, if the products of a farmer free of disease/residues. To be certain of results requires testing all animals or products; frequently an acceptable level of certainty can be achieved by testing only a subset of the animals/products. In this case, we use the following formula to calculate sample size:

$$n = \{1 - (\alpha)^{1/D}\}\{N - (D-1)/2\}$$

Where n is required sample size, αis 1-confidence level (usually α =0.05), D is estimated minimum number of diseased animals (population size*minimum expected prevalence), N is population size.

Example: Assume that you want to document the absence of Mycoplasma from a 200-cow herd and that based on your experience and the literature, a minimum of 20% of cows would have sero converted, if Mycoplasma was present in the herd.

- ✓ N = 200, α = 0.05, D = 40 (20/100*200)
- ✓ $n = \{1-(0.05)^{1/40}\}\{200-(40-1)/2\} = 13$

If you test 13 cows and get all negative test results, you can state that you are 95% confident that the prevalence of Mycoplasma in the herd is < 20%.

3.2.3 Sampling for Surveillance, Research and Outbreak investigations

Sampling for Surveillance; depending on the surveillance approach (risk based or randomized) used, both probability and non- probability type of sampling can be applied.

3.2.3.1 Risk based /targeted approach surveillance: this is conducted based on the increased likelihood of infection in particular localities or species. The purpose is to increase the probability of detecting the etiology or its antibody. Hence, in this case, the surveillance area (region, zone, district (woreda), kebele or village) is purposively selected. The primary sampling unit (herd) and individual animal (secondary unit) can be sampled using either simple random sampling or systematic sampling. In addition, depending on our sample size, we can use cluster type of sampling (sampling the entire herd) in sampling the primary unit (herd). Sometimes, if we think the probability of detection is not equal at a level of herd or individual animal, we can use purposive type of sampling on herds or individual animals, which are perceived to be at higher risk. The

sample size depends on the status of the disease. If it is exotic (new) disease, calculate the sample size using detection of new disease formula. If it is endemic disease, we can use the Thrusfield formula.

3.2.3.2 Randomized surveillance approach: in this case, the assumption is every animal in the population has equal chance of being selected. Selection of surveillance areas (region to village level) and herd as well as individual animal are randomly selected. However, it doesn't mean everything from top to dawn is random (random sampling type). We can use also non-probability type of sampling (purposive or convenience type) depending on the nature of the surveillance area and interest of the surveyor. Here to calculate the sample size, Thrusfield formula or other formulas taking in to account the herd number, herd size and expected prevalence of the disease in the selected surveillance area.

3.2.4 Sampling for Research

Sampling depends on the type of the research and nature of the research protocol. In experimental type of analytical research, similar sampling method is applied as in the randomized surveillance approach (as mentioned above). In case-control and cohort researches as well as other experimental type of studies, sampling depends on the interest of the researcher and research design. All animals selected to conduct the above type of researches are usually sampled (cluster type of sampling).

3.2.5 Sampling for outbreak investigation

The purpose of sampling during outbreak investigation is to detect the causative agent which causes the outbreak. There is no equal chance of detecting the etiology (cause of the outbreak) from all animals found in the outbreak area when we compared sick animal(s) with healthy animal(s) or affected kebele or village with outbreak free kebele or village. Hence, non- probability type of sampling (usually purposive type) which enables us to maximize the probability of detecting the cause is applied. With regard to sample size, of course, it depends on the type of sample, disease suspected, test method used and the quality of the sample. But we can sample up to 10 animals of the herd.

4. COLLECTION OF SAMPLES

4.1 Whole blood

- Aseptically bleed animals directly in to vacutainers containing EDTA or heparin preservatives;
- Mix gently by rolling the tube;
- Don'tfreeze but keep cool on ice (2-8^{o}C).

4.2 Serum

- Use plain vacutainer tubes for serology and mineral analysis;

- After collecting the blood sample, the tube is left untouched at room temperature till clotting is complete;
- The clot is then carefully removed;
- Centrifuge or allow any remaining cells to settle;
- Gently pour clear serum into a fresh or sterile tubes or cryo-vials;
- Care must be taken to keep the serum completely free from blood cells;
- Keep refrigerated (or at -20^{o}c) if the serumstays longer at your laboratory/clinic before shipment;
- Keep the sample cool on iceduring transportation to NAHDIC.

4.3 Smears

4.3.1 General points on how to prepare smears

- Use dry, non-greasy glass slides;
- Slide must be dried before wrapping in clean tissue paper;
- Protect from rain, flies, dust and abrasion;
- Submit at least 6 slides for a single case;
- Label smears clearly with pencil;
- Pack individually.

4.3.2 Blood Smears

- Remember to shake the blood gently while collecting blood to prevent clotting;
- Use clean, dry glass slide;
- Take a small drop ofblood and place on right end of one slide about 1 cm from edge;
- Place another glass slide (spreader) to the left of drop at 30^{o} angle gently touching the drop;
- Wait until blood has spread all way across the edge of the glass (spreader) slide;
- Move spreader slide to the left quickly but smoothly;
- Dry the smear by waving in the air;
- There are two type of smears.
  - Blood smear-for hematology as explained above;

  Capillaryblood smears-from ear, good for blood parasites: to obtain it, prick the tip of an ear with a needle, then, without pressing, bring a glass slide in contact with the slide. Use another glass slide to spread as described above.These can be thick or thin. It is preferable, if both typesare submitted.

4.3.3 Brain crush smears

- Take a match stick head size sample from grey matter; include small blood vessels (capillaries);
- Put material on a clean slide;
- Place a second slide on top of the brain material;
- Crush the sample between 2 slides and pull them gently away from on another length wise;
- Dry smears by waving them several times in the air;
- Label smears clearly with pencil;

- Pack individually.

4.3.4 Tissue impression smears

- Gently press a glass slide against a freshly cut surface of tissue to be examined;
- Dry the impression by waving in the air;
- Take necessary precautions to avoid cross contamination.

4.3.5 Pus Smears

- Prepare in the same way as tissue smears;
- If more than one day delay is expected in dispatch, fix by heating lightly with a match stick or lighter for less than 3 seconds;
- Label appropriately fixed smears.

4.3.6 Semen Smears

- Prepare in the same way as tissue smears.

4.4 Feces

- Should be fresh and free of soil and vegetation;
- If possible, collect directly from rectum or fresh feces from ground;
- Ideally, swabs of fresh feces or rectal/cloacal swabs should be collected for bacteriologicalexamination, and fresh bulk feces (with potassium permanganate preservative) for parasite examination;
- Keep the sample cool (2-8^{o}C) to prevent hatching of parasite eggs or bacterial overgrowth.

4.5 Skin scrapings

- Should be sent dry and unpreserved in a plastic bottle;
- Scrape deep at the periphery of the lesion until blood oozes;
- If ringworm is suspected, pull hair from edge of lesion with forceps and add to sample.

4.6 Skin biopsy

- Cut about 1 cm square and deep enough to reach subcutaneous tissue.
- Preserve in 10% buffered formalin for histopathology
- Use ice for skin biopsies to be screened for viruses e.g. LSD and Orf.

4.7 Urine

- Should be collected in clean and sterile bottles for bacteriologicaland hormone residues examination;

- 20ml urine + 1.5ml 10% formal saline for leptospirosis;
- 2.5% boric acid can be used as a preservative, if to be used for bacteriology;
- Use 10-50 ml falcon tube.

4.8 Pus

- Swab aseptically and deep from the edge of the abscess;
- Put swab in commercial semi solid transport medium;
- Collect into a sterile syringe or test tube and keep refrigerated.

4.9 Fluids

- This refers to fluids from joint, peritoneal, pleural, and pericardial cavities and cerebrospinal fluid etc.;
- Collect aseptically using a sterile needle and syringe/vacutainer;
- Should be preserved on ice.

4.10 Milk

- Ensure **NO** antibiotic treatment was given to the animal prior to sampling;
- Wash the teats with water;
- Wash your hands and disinfect with 70% alcohol;
- Dry off teats and disinfect with 70% alcohol;
- Initial stream must be discarded and then milk into a sterile bottle held almost horizontally to avoid dirt particles falling inside;
- Submit sample as fresh as possible keeping the cold chain.

4.11 Swab

- Collect specimen under good lighting and aseptic condtions.
- Pass the swab firmly over or into an area of suspected infection and
- Obtain a **s**ample of exudate, drainage, or purulent discharge if these are present.
- Return the swab to the transport tube and break the media ampule at the base of the tube to moisten the swab.
- Add a viral or bacterial transport media as deemed as necessary
- Submit sample as fresh as possible keeping the cold chain

4.12 Ruminal contents

- A large sample of about ½ kg is required;
- If plant poisoning is suspected, undigested leaves should be included;
- Keep cool on ice.

4.13 Aborted foetus, placenta, foetal fluids

- Preferably submit whole fetus or placenta preserved on ice;
- Do not freeze but keep cool on ice;
- If not possible, submit fetal stomach contents in a sterile universal bottle and fetal,lung, liver, spleen on ice and brain in 2.5% boric acid.

5. SAMPLES REQUIRED FOR LABORATORY DIAGNOSIS

The best sample to send to a laboratory is a live animal affected with the disease in question. The next best sample would be an animal that died recently showing signs of disease in question. It is of course, not always feasible to submit entire animals for laboratory examination.The third choice of samples are, therefore, those that are collected in the field from either live animals or during a necropsy.

The person who collects the samples should observe the following basic rules for proper sample collection.

- Sample diseased animals if possible;
- Sample lesions of suspect animals;
- Sample animals in early stages of a disease;
- Sample animals again 2-3 weeks later for a “convalescent” serum sample;
- Identify sampled animals properly.

For most animals ordiseases, the laboratory requires specific samples to be able to confirm the clinical diagnosis. In general, the under mentioned samples are also routinely submitted from categories of animals as indicated.

From live animals:

- Whole blood in EDTA, Heparin
- Paired serum samples
- At least 6 blood smears
- Feces
- Urine
- Swabs
- Skin scrapings
- Biopsies and impression smears
- External parasites

Certain infectious disease can be diagnosed by comparison of serological test result of 2 serum samples collected from the same animal 2-3 weeks apart. These are called “paired serum samples”

From dead or necropsied animals

- Smears from blood, brain, spleen, liver, lung *etc*; 6 smears from each organ;
- Liver, spleen, kidney, lung, lymph node and brain on ice or in 2.5% boric acid for bacteriology;

- Liver, spleen, kidney, lung, lymph node and brain in virus transport medium(VTM);
- Liver,spleen, kidney, lung, lymph node and brain in 10% buffered formalin for histopathology.

5.1 Appropriate samples by disease or conditions

Disease/condition	*Sample*	*Preservation/packing*	**Comments**
Abscess	Pus swabs deep into abscess	Semi-sold transport medium	Culture and sensitivity test take 7-10 days
	Smears of pus	Air dried	
Abortion	Whole fetus, placenta	Keep cool and submit quickly	Culture takes 7-10 days
	Cotyledons, fetal, liver, lung, Spleen	2.5% boric acid	
	Smears from stomach contents & cotyledons	Air dried	Serology takes 1-2 days
	Paired serum sample and milk from dam	Cool and rush to NAHDIC	
Actinobacillosis/ Actinomycosis	Pus swabs	Semi-solid transport medium	Culture and sensitivity test take 7-10 days
	Pus smears	Air dried smears	
	Biopsy of lesion	Formal saline	
African Horse Sickness	Whole blood in EDTA or Heparin	Keep cool on ice	**S**erology takes 1-2 days
	Paired serum sample	Keep cool on ice	Sero-typing referred to external labs
	Spleen, kidney, lymph nodes. heart, lung	Keep cool on ice/put in virus transport medium (VTM)	
Anaplasmosis	Blood smears from ear capillaries	Air dried	Test takes 1-2 days
	Whole blood (EDTA, Heparin)	Keep cool on ice	
Anthrax	Blood smears from peripheral vein	Wrap slides unfixed and unstained individually in tissue paper and pack between two card boards	Do not open carcasses suspected of anthrax
	Spleen smears, a swab of fresh long bone (do not open if strongly suspect anthrax)	Send in sealed metal container	
	Edematous areas are on neck and head	Send in sealed metal container	
Aspergillosis (Brooder pneumonia, Mycotic pneumonia)	Lung tissue/lesion from air sacs	Put in universal battle	Mycological culture
Babesiosis	Blood smears from ear capillaries	Air dried	Test takes 1-2 days
	Whole Blood (EDTA)	Keep cool on ice	
Blue tongue	Blood in heparin from acutely sick animals	Keep cool on ice	Sero-typing referred to external lab
	Paired serum	Keep cool on ice	Serology takes 1-2 days
	Affected tissues		
Bovine viral diarrhea	Paired serum sample	In semi-solid transport medium	Sero-typing referred to external lab

	Deep nasal and pharyngeal swabs Spleen and mesenteric lymph nodes	Keep cool on ice	Serology takes 1-3 days
Brucellosis	Serum	5 ml vacutainer tubes	*B. ovis*-CFT
	Milk	10 ml vacutainer tube + 0.5ml formalin	*B. abortus*-RBPT. CFT, ELISA
	Impression smears from placenta and lung	Submit smears unfixed and unstained Wrap slides in tissue individually, Put between cardboard	*B. melitensis*-RBPT, CFT, ELISA
	Smears from stomach of fetus	Air dried	*B. canis*-RBPT
	Smear from semen	Put ejaculate into test tube	Do not freeze serum, keep at 4^{o}C and do not cool serum for **B. canis**
	Fresh ejaculate	Keep cool on ice	All tests take 4-14 days
Calf diphtheria	Throat or mouth swabs	Semi-solid transport medium	Culture and sensitivity take 7 days
Campylobacteriosis (Vibriosis)	Sheath wash in 50 ml PBS	Keep cool on ice	Collect special medium from NAHDIC
	Insemination straws	Keep in Liquid nitrogen	Wash sheath vigorously 10x, avoid sunlight, deliver to lab within 6-8 hours of collection and do not freeze; Test *takes 7 – 10 days*
Canine distemper	Whole blood (EDTA, Heparin)	Keep cool on ice	From dead and live animals
	Conjuctival scrapings on slides	Air dried on microscope slides	For IFAT
	Lung impression smears, tonsils	Air-dried and unstained	From dead animals
	Brain, liver, Lung, Urinary bladder	Fix in 4% buffered formalin	Referred to external labs
Caseous lymphadenitis	Pus swabs	Semi-solid transport medium	Culture & sensitivity take 7-10 days
	Smears	Air dried	
Chlamydiosis	Fetal tissue, placenta, joint fluid, eye swabs (microscopy)	Swab in chlamydial transport medium, tissues in separate sterile containers, keep cool and rush to NAHDIC	Microscopy takes 1-2 day
	Impression smears from above	Wrap slides individually in tissue paper between cardboard	Serology takes 1-2 days
	Serum for serology	Keep cool	Chlamydiosis in birds
Coccidiosis	Intestinal scrapings, feces	Make smears during sampling	Direct smear
Contagious bovine pleuro pneumonia (CBPP)	Fresh lung with lesions (Eg. sequestra and marbling)	Ice and some in 10% formal saline	Use Rayon swabs with plastic shafts and never freeze samples for culture of Mycoplasma species
	Pleural fluid	Keep cool and rush to the lab	Tests take 4-21 Days
	Serum from in-contact animals	Keep cool	ELISA takes 1-2 days

	Nasal swabs	Special transport medium (Rayon swabs) with plastic shafts	CFT takes 1-3 days
Dourine	2ml serum samples Vaginal and perpetual wash	Keep cool on ice	
E.coli (calf diarrhea)	Feces (diarrhea), tissues (liver, intestine, heart, kidney)	Take with universal battle and put refrigerated	Bacterial culture and Omnilog takes up to 7 days
Enterotoxaemia	Fresh whole carcass Small intestine	Keep cool on ice	Serology takes 1-2 days Culture and sensitivity take 7-10 days
	Urine		Urinalysis for glucosuria takes 1-2 days
Erysipelosis (Swine)	Blood in heparin	Keep cool on ice	Culture and sensitivity test take 7-10 days
	blood smear	Air dried	
Equine infectious anaemia	Paired serum samples Whole blood (EDTA, Heparin) Deep nasal or pharyngeal swabs	Keep cool on ice Sterile phosphate buffered saline at 4^0C	Referred to external labs From acute cases only
Fascioliasis	Fecal sample Liver sample	Keep cool on ice 10% buffered formalin	Fecal analysis takes 1-2 day Histopathology takes 1-3 days
FMD	Tongue epithelium Swab	Submit samples as instructed by NAHDIC	 For surveillance test takes 1 to 2 days
	Vesicular fluid/probang		For genotyping referred to external labs
Fowl typhoid	Liver, spleen and reproductive tract (carrier stage)	Put in universal battle in aseptic condition	Bacterial culture and Omnilog takes up 7days
	Tissues from all organs and faeces (acute stage)	Put in universal battle in aseptic condition	Bacterial culture and Omnilog takes up 7days
Goat Pox (same as sheep pox)	Skin swabs/scabs Skin biopsy in 10% buffered formalin Paired serum sample	In a sterile tube on ice	Tests take 14-21 days
Q fever (Coxiellosis)	Blood serum Milk, Urine	Keep cool on ice Put in universal battle and Keep cool on ice	ELISA takes 1-2 days Bacterial culture and Omnilog takes up 7days
	Swab	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
Heart water	4 Brain crush smears Grey matter	Air dried Keep cool on ice	Test takes 2-3 days
	Swabs from upper respiratory	Put in universal battle with Virus trans	PCR test takes 1-2 days

Infectious bronchitis	tract	port media(VTM) and keep cool in ice	
	Tissues (tracheal mucosa, lung, kidney, oviduct, caecal tonsils of the intestinaltract, proventriculus	Put in cur-vial tube and make refrigerated	Serology takes 1-2 days
	Blood serum		
Infectious Bursal Disease	bursa Fabrici	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
	Blood serum	Put in cur-vial tube and make refrigerated	Serology takes 1-2 days
Infectious bovine rhinotracheitis	Deep nasal swabs	Semi-solid transport medium	Only from acute cases, labile virus isolation & serology referred to external lab
	Paired serum sample	Keep cool on ice	
Infectious laryngotracheitis	Swabs (tracheal, oesophageal, conjunctival)	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
	Tissues (head, neck, (trachea, larynx)	Put in cur-vial tube and make refrigerated	Serology takes 1-2 days
	Blood serum	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
Johnes Disease (Paratuberculosis)	Fecal samples	Keep cool on ice	Scrap gently to collect some mucosa of the rectum, do not freeze, culture takes 3 weeks and serology takes 3-4 days
	Rectal scraping	Keep cool on ice	
	Pieces of affected intestine	Part in10% buffered formalin	
Listeriosis	For CNS signs: brain stem, upper spinal cord, CSF (on ice)	Ice or 2.5% Boric acid and some in 10 % buffered formalin	Culture & sensitivity test take 7-10 days
	Abortion	Ice or 2.5% Boric acid and some in 10% buffered formalin	Histopathology takes 1-3 days
	Whole fetus or fetal internal organs		
Lumpy skin Disease	Small skin biopsies, Skin nodules, lung, lymph nodes	½ biopsy on ice and ½ in formalin	Isolation referred to external lab and serology takes 1-2 days
Malignant Catarrhal Fever	Vacutainers of whole blood in heparin for DNA hybridization/ PCR	Keep cool on ice	
	Spleen, lymph nodes	Keep cool on ice	Histopathology takes 8-12 days
	Liver, kidney, brain and small intestine	Transport medium some in 10% buffered formalin	Referred to external lab
	Nasal/ eye swabs	Transport medium	
	Serum		
Mange	Skin scrapings and scabs	Clean sterile container	Deep and at periphery of lesion, test takes 1-2 days

	Ear wax, if external ear canal is affected		
Marek's Disease	lymphomas, spleen , feather tips	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
	blood serum	Put in cur-vial tube and make refrigerated	Serology takes 1-2 days
Mastitis	Fresh milk samples (individual quarter sampled differently)	Milk mixed with 2.5% boric acid, fresh milk in sterile container	1 Volume of milk + 4 volumes 2.5% boric acid, milk kept cool, culture and sensitivity test take 7-10 days
Mycoplasmosis (Chronic Respiratory Disease)	Lung tissue/air sac lesion	Put in universal battle in aseptic condition	Bacterial culture and Omnilog takes up 7days
Mycotoxicosis	500g feed	Well tied off in plastic or cardboard box	Original batch of feed
	Liver	10% buffered formalin	For aflatoxin assays, samples have to be accumulated, tests takes 21-30 days
Newcastle Disease	Swab (tracheal or oropharyngeal and cloacal swab)	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
	Tissue (lung, intestine, brain , caecal), Tissue pool of (tonsils kidneys, spleen, liver and heart)	Put in universal battle with Virus trans port media(VTM) and keep cool in ice	PCR test takes 1-2 days
	Blood Serum	Put in cur-vial tube and make refrigerated	Serology takes 1-2 days
Pasteurellosis (Septicaemia A)	Blood in heparin	Keep cool on ice	Only acute case
	Heart, lung, liver, spleen	Keep cool on ice or 2.5% boric acid	Culture and sensitivity test take 7-10 days
	Blood and impression smears of above	Dry	
Pasieurellusis (Pneumonia B)	Deep nasal swabs- from live animals		Culture and sensitivity test take 7-10 days
Pullorum Disease (S.pullorum)	ova and oviduct (carrier stage)	Put in universal battle in aseptic condition	Bacterial culture and Omnilog takes up 7days
	Tissues from all organs and faeces (acute stage)	Put in universal battle in aseptic condition	Bacterial culture and Omnilog takes up 7days
Rabies	Cut brain in half from front to back	Put half into buffered formalin, rest in 50% glycerol saline	Mark container Rabies
	Lungs and trachea-from dead animals	Keep cool on ice or 2.5% Boric acid	FAT takes 1-2 days, biological assay takes 30 days
Rift Valley Fever	Paired serum samples (2 ml serum)	Keep cool on ice	Serology takes 1-2 days
	Blood in heparin	Keep cool on ice	
	Liver, spleen, kidney & heart	10% buffered formalin	

Ringworm	Scrape skin and hair on periphery of lesion	No preservative, enclose in envelope or purple top bottle used for fecal samples	Potassium hydroxide digestion test takes 2- 3 days, isolation of fungus takes 21-30 days
Dermatophilosis (Streptothricosis)	Skin scabs	Make impression smears on glass slides, air dry smear, wrap each slide individually	Test takes 7-10 days
Sheep Pox	Skin scabs for electron microscopy	Keep cool on ice	Tests take 14-21 days
Strangles	Nasal swabs or discharges		Culture takes 7-10 days
	Pus smears	Air dried smears	
Toxoplasmosis	Paired serum samples from dam	Keep cool on ice	Serology takes 1-2 days, biological testing takes 8-9 weeks
	Brain, lung, placenta and kidney	10% buffered formalin	
	Entire lamb or kid, placenta	Keep cool on ice	
	Fetal brain, lung, liver and kidney	On ice and 10% buffered formalin	
Trichomoniasis	Preputial washings	See for campylobacteriosis	Microscopy takes 1-2 days, culture takes 11 days
Trypanosomosis	Ear capillary blood smears	Air-dried capillary smears to be made on consecutive days	Testing takes 1-2 days
	Thick blood smears		
Theileriosis (cerebral)	Ear capillary smears/L. node aspirate	Air dried	Specifically for cerebral theileriosis
	Whole blood in EDTA/Serum	Keep cool on ice	DNA detection studies
	Brain smears from lesion	Air dried	Blood to reach OVI within 24 hours of collection
	DNA hybridization: 10 ml blood in heparin	Keep cool on ice at 4^{o}	Testing takes 1-2 days
Ulcerative Lymphangitis of horses and cattle	Pus swabs	Semi-solid transport medium	Notifiable disease, inform DAHP, test takes 7- 10 days

5.2 Entomology samples

Sample	***Preservation and packaging***	**Comments**
Live ticks	Glass/plastic vials plugged with cotton wool and securely packed in sealed cardboard box	Collect the ticks with forceps while wearing gloves, keep sample from each different host separate, moist tissue paper in the vials
Dead ticks	Sealed glass/plastic vials containing 70 % alcohol or 5% formalin and securely packed in sealed cardboard box	Keep samples from each host separate, identification time is 3-5 days

Mosquitoes	Between thin layers of tissue paper in cardboard box or double pinned insample box	Identification time is 3-5 days
Flies, fleas, lice and gnats/midges	Sealed glass/plastic vials containing 70% alcohol and securely packed in sealed cardboard box	Identification time is 3-5 days
Maggots	Sealed glass/plastic vials containing 70% alcohol and securely packed in sealed cardboard box	Kill the maggots by placing in hot tap water, take samples from surface and depth of wound, identification time is 5-7 days
Skin scrapings for scab, mange mites and ringworm	Sealed glass/plastic vials securely packed in sealed cardboard box or dry in envelope	Make deep skin scrapings (dry or after application of liquid paraffin) of the affected area with sharp blade, if possible also send biopsy material for histopathology, test time is 2-5 days

5.3 Haematology samples

Procedure/ parasite	***Sample***	***Preservation and packaging***	**Comments**
Nematode and trematode egg counts and larval cultures	30-50g fresh feces	Fill up sample container to exclude air and keep cool on ice	Do not freeze the samples, test time is 2-3 days
Worm counts (worm burden)	Unopened organ or intestinal tract	Freeze or cover the organs with 10% formalin in thick sealed plastic bag or suitable container	Test time is 7 days
Worm identification (adults or larvae)	Fixed worms	70% alcohol and place in suitable container.	Specific identification of unusual parasites may require submission to a reference laboratory, test time is 3-7 days
Canine and feline filariasis (identification of microfilaria like Dirofilaria and Dipetalonema species)	Blood in EDTA or heparin	Keep cool on ice	Test time is 1-2 days
Parafilaria	Dry blood from bleeding point	Suitable container, e.g. test tube	Teat time is 2-3 days

5.4. Differential diagnoses for specific syndromes

Presenting problem	**Possible causes**
Infectious abortion	Chlamydiosis, IBR, leptospirosis, brucellosis, wesselsbron disease, salmonellosis, Q-fever, fever reaction, toxoplasmosis, BVD, listerosis, akabanediseases, campylobacteriosis, neosporosisetc.
Non-infectious abortion	Prostaglandins, corticosteroid , trauma ,Stress, fever, poisoning, malnutrition etc.
Male infertility	Failure to serve due to low libido Failure to mount due to laminitis, footrot, overgrown hooves, arthritis, weak pasterns, back pain Failure to serve properly (no intromission) due to balanoposthitis, phimosis, persistent frenulum, penile haematoma, shortpenis Failure to thrust, stress, overweight, back pain, prolapse of the prepuce Return to service of females due to poor sperm quality, overuse of billy goat/bull/stallion/boar/ram, age, nutrition,, disease, abnormal testicle, abnormal epididymis, abnormal accessory sex glands
Weak newborn animals	Premature birth, low birth weight, birth injury,malnutrition, infection and cold exposure (hypothermia)
Infectious causes of lameness	Infectious diseases of foot, inter digital dermatitis, foot rot, foot abscess, dermatophilosis, foot and mouth disease
Non-infectious causes of lameness	White line disease, bruising/trauma to the hoof,foreign body,fractured pedal bone,laminitis, congenital abnormalities (newborn animals)
Chronic weight loss	Primary nutritional deficiency, inability to utilize available foodstuffs, unwillingness to feed on available feedstuff, inability to increase feed intake to match production demands, interference with absorption of nutrient or loss of nutrients due to diarrhea, rumen or intestinal immobility, presence of chronic disorders e.g. TB,CBPP, trypanosomosis, pruritic conditions interfering with feeding
External swellings	Throat swellings, thymus enlargement, thymichyperplasis/non regression of thymus,thymoma fat in the thymus, wattle cysts salivary cysts, thyroidenlargement (goiter), abscess on the head and neck, vaccination reactions, toothrotabscess, bottlejaw, impactedcud, bighead, tumors of herpus virus, dermatophilosis, ringworm, actinomycosis, abscess on the body, haematoma/seroma, tomour on the body, prolapse, umblicalhernia, ectopicmamamry tissue, pre-parurientedema, LSD/pseudo-LSD, tick bite allergy, kedscyst and warbleflies, orf, chorioptic mangeLeg swelling
Skin diseases	Pruritic skin diseases: lice, sarcoptic mange, fly worry, harvest mites, forage mites, psoroptic mange, *Strongyloidespapillosus*, Onchocercosis, *Pemphigus foliaceus*, photosensitization Non-pruritic diseases: chorioptic mange, demodectic mange, *Staphylococcal dermatitis*, orf, zinc deficiency, urine scald, alopecia/hyperkeratosis in older bucks, trauma, labial dermatitis in kids, ringworm, mycotic dermatitis, dermatophilosis, iodine deficiency, selenium/vitamin-E deficiency
Nervous diseases	Neonatal animals: congenital infections, hypoglycaemia, birth trauma, enzootic ataxia (sway back) Young animals: spinal abscess, trauma, congenital vertebral lesions, tick pyaemia, bacterial memingitis, focal symmetrical encephalomalacia (enterotoxoaemia), tetanus, disbudding memingoencephalitis, caprine arthritis, encephalitis 2-7 months: trauma, delayed swayback, spinal abscess, coccidiosis, heart water 7 months-adult: infectious diseases (heart water, listeriosis, tetanus, CAE, rabies, spongiform encephalitis), metabolic diseases

	(Ketosis, cerebrocortical necrosis (CCN. polioencephalomalacia), hypocalcaemia (milk fever), hypomagnesaemia (grass tetany), transittetany, periparturienttoxaemia, space-occupying lesions in the brain (cerebral abscess, coenuriasis (gid), pituitary abscess syndrome),*Oestrusovis*, space occupying lesions of the spinal cord (spinal meningitis,spinal abscess, vertebral osteomyelitis, tumour, vestibular disease), otitis media/internal, ear mite infection, hepatic encephalopathy, poisonings (lead or plant-Solanum spp, organophosphates, rafoxamide, urea)
Diarrhoea	Birth to 4 weeks: nutritional, enterotoxigenic *E.coli*, Salmonella spp, *Clostridium perfringens* type C, *Clostridium perfringens* type B, *Campylobacter jejuni*, rotavirus, coronavirus, cryptosporidium, *Strongyloides papillosus* 4-12 weeks: parasitic gastroenteritis, coccidiosis, *Clostridium perfringens* type D, salmonella, giardiasis, nutritional factors, *Campylobacter jejuni*, toxic agents Over 12 weeks: parasitic gastroenteritis, coccidiosis, *Clostridium* perfringens type D, salmonella, nutritional factors, toxic agents, liver disease, copper deficiency, John's disease
Colic in adult animals	Diarrhoea/enteritis, indigestion (ruminalatony), acidosis (ruminal impaction), ruminaltympany (primary(frothy bloat), secondary (choke), enterotoxaemia (*Clostridium perfringens* type D), abomasal ulceration, left-sided displacement of the abomasum, cryptochid, urolithiasis, liver disease, toxic minerals (e.g. copper, lead, fertilizer ingestion), post-parturient problems, metritis, retained fetus membrane, cystitis, plant Poisoning
Colic in newborn animals	Diarrhoea, abomasal bloat, mesenteric torsion, coccidiosis, ruminal bloat, constipation, *Clostridium perfringens* type D, urolithiasis, visceral cysticercosis, plant poisoning
Respiratory distress	Infectious diseases: bacterial (pasteurellosis, *Corynebacteriumpyogenes*, Staphylococcal spp, Streptococcal spp, Haemophiluspp, *Klebsiellapneumoniae*, tuberculosis, mycoplasma), viral (herpus viruses like IBR (BHVI), respiratory syncyital virus, parainfluenza virus 3, caprine arthritis, encephalitis, pulmonary adenomatosis), parasitic (*Muelleriuscapillaries*, *Dictyocaulusfilarial*, *Protostrongylusrufescens*, hydatidcysts) Air was obstructions: foreign body, lymph node enlargement, tracheal collapse, tumour, inhalation, pneumonia, trauma, heat stress, allergic alveolitis, neoplasia
Anemia	Helminthiasis (haemonchosis, trichostrongylosis, fascioliasis), protozoa (coccidosis, eperythrozoonosis, *Theileriamutans*), bacterial (lptospirosis; rickettsialanaplasmosis), plantpoisoning (bracken fern (*Pteridiumaquilinum*), pyrrolizidine alkaloid poisoning, Brassica spp, onion), external parasites (ticks, sucking lice, keds), mineral deficiencies (copper (or molybdenum excess), cobalt), mineral excess (copper), protein deficiency (primary or secondary), choronic diseases (John's disease, tumour)
Sudden death in young animals	Hypothermia/hypoglycaemia, abomasal bloat, ruminal bloat, mesenteric torsion, disbudding meningoencephalitis, anaphylactic shock, plant poisoning (cardiac glycoside), chemical poisoning, white muscle disease, septicaemia, coccidiosis, diarrhea caused dehydration, enterotoxaemia, listeriosis, pasteurellosis, haemonchosis, salmonellosis
Sudden death in adults	Heart water, trauma, ruminal bloat, snake bite, acidosis (ruminal impaction), lightning, cold stress, anaphylactic shock, plant poisoning, chemical poisoning, hypomagnesaemia, transit tetany, gangrenous mastitis, enterotoxaemia, listeriosispasteurellosis, haemonchosis, acute fascioliasis, anthrax, blackleg, botulism
Eye diseases	Infectious keratoconjunctivitis, foreign body trauma, entropian, photosensitization, blindness (urea and lead poisoning), avitaminosis A, moon blindness in horse

6. REFERENCES

- World Organization for Animal Health. Manual 6- Sample collection and transport
- OIE Terrestrial Manual 2008 Chapter 1.1.1 Collection and shipment of Diagnostic Specimens
- OIE Guidelines for animal disease control. May 2014
- OIE Terrestrial Manual 2013 Chapter 1.1.1 Sampling Methods

7. ANNEXES: SUBMISSION FORMS, CONTACT ADRESSES, PRIORITY DISEASE

Annex-1 LABORATORY SAMPLE SUBMISSION FORM (NAHDIC-FRM-GEN-006-4A)

National Animal Health Diagnostic and Investigation Center (NAHDIC)
Quality Management System
Laboratory sample submission form

For receptionist use only
Submission No: ……..
Date: ……...
Time: …….

Data to be completed by the sample submitter

Submitter's s
Name: …… **Qualification:** ……..… **Sign:** ……..
Sender Type: Organization [ ] /Institute [ ] /Private Owner [ ]
Name…….
Sample Address: Region ……Zone ……District ……..……
Farm/PA/kebele ……..…. Tel …… Fax …….
E mail ……..…… P.O.Box ……
Date sampled: …… Geo. Ref: N ……..... E ……..……... Altitude ……....
Sampling Purpose: Outbreak investigation (Ou) [ ] Surveillance (Su) [ ] Certification (Ce) [ ]
□ Research (R) [ ] Export (Ex) [ ] Diagnosis (Di) [ ] Others ……
Sample type /Number: Serum (S) [ ]/……..Tissue (T) [ ]/……. Swab (Sw) [ ]/…
Whole blood (Whb) [ ]/……Faeces (F) [ ]/…….....
Smear (Sm) [ ]/……..... Scraping (Scr) [ ]/…….
Ectoparasites (Ecp) [ ]/…… Others …….
Preservative used: …… Type of transportation employed:…….
Species……
Breed: Local □[ ] Exotic □[ ] Cross [ ]□ Mixed [ ]□
Production system: Extensive [ ] Intensive [ ] Backyard poultry [ ] Non domesticated [ ]□
Case history (for outbreak, incidental cases)
Age group affected…….. **Number sick**……..… **Number Dead**…… **Number atrisk**……...

Major clinical signs: …….....
…….....
……...
Duration of the outbreak: …….....
Postmortem findings: …….....
…….....
…….....
Disease suspected/Disease to be tested: …….....
……
Measures taken and response: ……..
…….....
…….....
Vaccination history: …….....
……
Fee Applicable [ ] not applicable [ ]

Note: Specimens for surveillances and certification please fill part II page 3 0f 4 of this form additionally **Sample Reception (SR)**

For sample reception use only

Sample acceptance or rejection (please tick [x] for incorrect and [√] for correct or write your opinion under each condition)							
Document accompanying the samples	**Container condition**	**Labeling**	**Transport condition or preservation**	**Volume of sample**	**Sample type**	**Number of sample(s)**	**Decision fit /unfit for test required**

Remark……

……....

Lab code……. **Sample submitted to:** Bacteriology [ ] Helminthology [ ]□ Pathology [ ]□ Pharmacology &Toxicology [ ] □ Protozoology [ ]□ Bacterial [ ] Serology [ ]□ Viral Serology [ ]□ cell culture [ ]□ Molecular [ ] Acarology /Entomology [ ]□

Sample Receptionist Name: …….. **Signature:** …….. **Date:** …….

Duty Veterinarian Name: …… **Signature:** …..…… **Date:** …….

Submitted to the lab Date: …..……**Time**……..…**Received by** (name/signature)……

For laboratory use only

Sample type	**Number of Sample(s)**	**Type of test /Examination**	**Date tested**	**Remark**

Laboratory findings: …….
…….
…….

Conclusion and Recommendation: ……..
…….

…….
…….
Examined by……. **Signature**…….. **Date**……..……..

Laboratory head……..…**Signature**…… **Date**……

Result received by reception unit
Name…….……..**Signature**……**Date**……**Time**……..

Part II: additional information to be filled for Outbreak, Surveillance and Certification Purpose

Animal ID	Kebele	District	Species	Sex	Age	Sample type	Geo reference		Altitude	Lab. code	Date tested	Test Type	Test result
							N	E					

Tested by……... **Signature:** …… **Date:** …… **Time:** …….

Result received by…….**Signature:** ……. **Date:** …….. **Time:** ……

Annex 2: Contact Address of NAHDIC Staff during Sample collection and Submission

Name	**Position**	**Phone**	**Email**
Mesfin Sahle Forsa(Dr.)	Center Director	+251911933248	sebeta.mesfin@gmail.com
Solomon Gebre (Dr.)	Diagnostic Core Process Owner	+251 911453760	gebredufe@yahoo.com
Abera Kebede	Quality Manager	+251 0911949928	melatkeb@yahoo.com
Hagos Asgedom (Dr.)	Sample Reception Head	+251 964582863, 01118334991	haghad2012@yahoo.com
Fasil Aklilu (Dr.)	Viral Serology One Head	+251 918752816	fasil.aklilu@yahoo.com

Annex 3. List of priority zoonotic diseases in Ethiopia (using a one health approach)

Rabies 1
Anthrax 2
Brucellosis 3
Leptospirosis 4
Echinococcus 5

Source: Prioritizing zoonotic diseases in Ethiopia using a one health approach, journal homepage: www.elsevier.com/locate/onehlt

Annex 4: List of 15 prioritized diseases in their ranking order

No	Disease	Score	Remark
1	PPR	7.83	The list is subject to continuousrevision and should be updated atcertain period of time interval.Regardless of the score, RVF shouldbe one of priority diseases ofimportance as there is HIGH risk ofintroduction.
2	FMD	7.235	
3	Anthrax	7.23	
4	Brucellosis	6.93	
5	LSD	6.69	
6	Rabies	6.02	
7	BTb	5.82	
8	SGP	5.36	
9	Trypanosomosis	5.34	
10	Ectoparasites	5.237	
11	AHS	4.93	
12	NCD	4.9	
13	Rift valley fever	2.6131	
14	Mastitis	2.06	
15	CBPP	1.87	

Source: National priority animal diseases of socio-economic and trade significance, including those transmissible to humans:Federal Democratic Republic of Ethiopia Ministry of Livestock and Fisheries, Epidemiology Directorate,June 2016, Addis Ababa

Annex 5: Materials required for sample collection

- Plain Vaccutainer tube and
- Heparinized/EDTAvaccutainer tube
- Sampling needle (with different gauges),
- Needle holder
- Crovial tube (for serum)
- Wide gauge needle for fluid/abscess collection
- Postmortem kit (knife, scalpel handle, blade, scissors, forceps, saw…)
- Sterile plain swabs with transport medium
- Probang cup (for collection of retropharyngeal fluid for FMD)
- Universal bottle
- Universal bottle with transport media for tissues intended for Cell culture & PCR tests
- Universal bottle with 10% buffer formalin for tissues intended for histo-pathology test
- Universal bottle with ethanol 70% for tick, fly, mosquitoes, flees and lice collection.
- Permanent marker for labeling
- 70% ethyl alcohol (disinfectant)
- Personal Protective Equipment(PPE)
- Glove
- Cotton and gauze,
- Glass microscope slide with cover slip
- Rope (for restraining)

Annex 6: Pictures of some sample collection materials

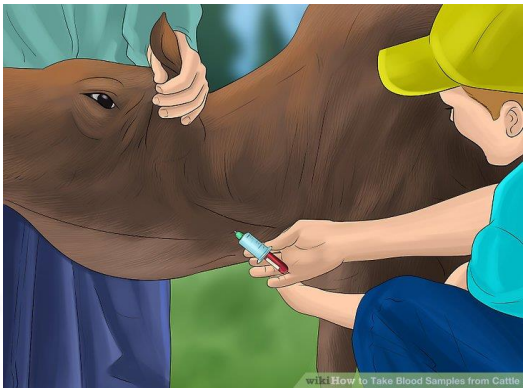
EDTA vacutainer tubes and racks

Plain vacutainer tubes

Vacutainer tube holder

Cryogeneic vials with racks

Needle for collecting blood sample



How to take blood sample from cattle