NATIONAL GUIDELINES FOR ONE-HEALTH SPECIMEN COLLECTION AND HANDLING

First Edition

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Foreword

Laboratories are integral to any healthcare delivery system. They help with detection , identification and reporting of all diseases of public health importance. Strengthening laboratory services is one of the core capacities for IHR 2005 framework , One health and Global health security Agenda. The Ministry of health and Ghana Health Service's aim of achieving high quality curative and preventive care services means laboratory services also need to be strengthened. Having quality samples being submitted for testing at the different points of testing coupled with an effective sample transportation system are key in this endeavor.

The development of this guideline adopting a one health approach is a fundamental step in ensuring provision of sustainable and consistent lab services through all tiers of the healthcare system. This document will serve as the guiding principle for the delivery of quality samples in a one health approach. This guideline is a result of a consultative process involving Ministry of Health, Ghana health service , Veterinary services and the environmental health departments. This guideline aims to provide guidance for proper collection and handling of samples encompassing a one health approach.

The Ministry of health and Ghana Health service invites all stakeholders to internalize and to embrace the use of this document for collection and handling of samples. Together with the sample referral and transport system, this is key to sustaining consistent provision of laboratory services to all who require it.

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Table of contents

List of figures

List of tables

List of acronyms/Abbreviations

AFB	Acid fast bacilli
ART	Anti-Retroviral Therapy
AU-IBAR	African Union Intra-Bureau of Animal Resources
BAL	Bronchoalveolar Lavage
CDC	US Centers for Disease Control and Prevention
CFT	Complement Fixation Test
COVID-19	Coronavirus disease
CSF	Cerebrospinal fluid
EDTA	Ethylenediaminetetraacetic Acid
EID	Early Infant Diagnosis
ESR	Erythrocyte Sedimentation Rate
FAO	Food and Agriculture Organization
GHS	Ghana Health Service
HCV	Hepatitis C Virus
HLA	Human leukocyte Antigen
HI	Hemagglutination Inhibition Assay
HIV	Human Immunodeficiency Virus
IHR	International Health Regulations
IATA	International Air Transport Association
IQLS	Integrated Quality Laboratory Services
JEE	Joint External Evaluation
LP	Lumbar puncture
MOA	Ministry of Agriculture
MOH	Ministry of Health
OIE	World Organization for Animal Health
PCR	Polymerase Chain Reaction
PCV	Packed Cell Volume
PVA	Poly Vinyl Alcohol
PPE	Personal Protective Equipment
RDT	Rapid Diagnostic Test
SOP	Standard Operating Procedures
SRN	Sample Referral Network
SST	Serum Separating tube
TB	Tuberculosis
WHO	World Health Organization

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Definitions of terms

Packaging: It includes the receptacle(s) and other components or materials necessary for the package to perform its containment function in support of the completed product prepared for transportation with the regulation's minimum packing requirements

Rejection Criteria: A set of requirements or preconditions standard (principle) to differentiate qualified specimens during the time of specimen accepting procedure to achieve quality result in Laboratory testing process

Referring Laboratory: A Health Facility that sends specimen for Laboratory testing or further investigation purpose to other Health Facility based on the available healthcare delivery tiered system.

Referral (Receiving) Laboratory: A Laboratory that received specimen for examination or further investigation analysis through the integrated Laboratory tiered structures healthcare delivery system.

Specimen: Are human or animal materials, collected directly from humans or animals, including, but not limited to, excreta, secreta, blood and its components, tissue and tissue fluid swabs, sputum, urine, blood, surgical drain fluid and body parts being transported for purposes such as research, diagnosis, investigational activities, disease treatment and prevention.

Triple Package: For transporting diagnostic specimens & biological agents based on National/International accepted regulation system in tri part specimen container mechanism by once; that includes three distinct layers of protection Primary receptacles, Secondary packaging and Outer packaging.

1 Introduction and background

Ghana has made several efforts to adopt and implement the one-health and integrated disease surveillance and response. These efforts include the establishment of national committees such as One-health National Technical Coordinating Committee, IHR 2005 One-Health Technical Committee, One-Health AMR Technical Committee, One-Health Emergency Response Team. These committees were coordinated by the Ghana Health Service under the Ministry of Health for over a decade now. However, these committees operated mainly as ad-hoc committees which lacked consistent funding arrangements.

A one-health approach enables Ghana to build systems that enable earlier detection of emerging threats to human, animal and environmental health and for mobilizing interventions to mitigate their threats and spread. A one health diseases prioritization meeting was held in 2018 to prioritize zoonotic diseases in the country. This resulted in 15 diseases being prioritized namely Influenza, Polio, HIV/AIDS, TB, Malaria, *Salmonella enterica* serotype Typhi induced diarrhoea, Ebola, Cholera, Meningitis, Rabies, Yellow Fever, Measles, Rubella, Anthrax, and Hepatitis).

It is important to note that 60% of human infectious diseases worldwide are caused by pathogens of a zoonotic nature with most originating from wildlife and having serious consequences for livestock. This makes timely diagnosis of animal diseases an important part of human health. Diseases like severe acute respiratory syndrome (SARS), highly pathogenic avian influenza and pandemic influenza are examples of how disease events can turn into major outbreaks or pandemics with significant impacts on public health, animal health and economies. Many other zoonotic diseases including neglected diseases may be more limited in terms of rapid spread but strongly affect human and animal health, production capacity, value chains and trade and livelihoods. [1]

New threats related to climate change, food safety and chemical hazards also pose a complex set of challenges involving human, animal, and environmental health. The one health approach promotes cooperation and coordination for disease surveillance, outbreak investigation and response activities from different fields. This guide aims to describe collection and handling of human, animal and environmental samples with the end goal being to improve quality of samples delivered at the different points of testing in the country. This will in turn in turn improve timeliness of testing and diagnosis of potential disease threats.

2 Purpose of the guidelines

This document aims to:

- Provide guidelines and instructions for the proper collection and handling of samples to be implemented by the one-health system stakeholders that collect and send samples to testing laboratories.
- Improve the quality of specimens collected and handled.
- Ensure safety and security of all personnel, individuals as well as the environment that are involved in specimen collection and handling.

[1] WHO-OIE Operational framework for good governance at human-animal interface: Bridging WHO and OIE tools for assessment of national capacities , 2014

3 Target users of the guidelines

The guidelines target a range of stakeholders who are involved in specimen collection and handling.

These target users include the following:

- Human and animal healthcare workers
- Environmental, water and food testing laboratory personnel
- Policy makers and administrators of health facilities and districts

4 Human specimens

4.1.1 General human specimen collection and management guidelines

4.1.2 Request form and completion

The following essential information must be documented in a legible manner on the request form for the hospital:

- Patient's unique clinic/hospital number.
- Patient's first name and surname
- Patient's Date of Birth (DoB) / Age
- Patient's gender
- Name and signature of requesting clinician
- Requesting site Information (Site name and site code).
- Tests requested
- Date and time of sample collection
- Date and time of specimens submitted to the laboratory
- Sample type
- Clinical summary

Relevant clinical information/data appropriate to the test(s) requested must be supplied.
For disease surveillance or other priority diseases, disease specific request form or case-based forms should be used.

4.1.3 Patient identification

For verbal identification:

- Greet the patient and identify yourself.
- Always ask the patient to state his/her full name. Never ask by name e.g., "Are you Melvin Kwadwo Amanfo?"
- If the patient is unconscious, ask the relative/ caretaker for the patient's full name.
- Ask the patient's date of birth and ask them to spell their names if there is the need to query the patient's identity.

Examination of any of the following should follow verbal identification to confirm the patient's name, hospital number and date of birth: ID card, Hospital/clinic card/book or other relevant documents.

4.1.4 Specimen collection room or area

Specimen collection room or area should be well be equipped and arranged to ensure the safety and comfort of the client and the health worker:

- Specimen collection should be performed in a clean, quiet, private, well-lit and ventilated environment
- Client sample collection facilities should be separated from reception/waiting areas to ensure patient privacy
- Specimen collection areas should be separated from analytical areas
- Always work on **one patient at a time** no matter how crowded
- Dedicated venous blood collection chairs and/or bed should be in place as well as a chair for the phlebotomist. The armrests of the chair should be adjustable to enable the optimum position for blood collection to be obtained. If a dedicated venous blood collection chair is not available, the chair must have arm rests to prevent clients from falling if they feel dizzy, weak, become unconscious or aggressive.
- Equipment and supplies should be available in sufficient quantities and appropriate for their intended use
- Hand sanitizing or washing areas with soap and/ or appropriate sanitizers and paper towels should be available and accessible to ensure proper hand hygiene.

4.1.5 Collecting the specimen

Specimens should be collected according to the instructions set out in the appropriate sections in this document or in specific laboratory SOPs.

4.1.6 Labeling of primary specimen containers

Samples shall be properly labeled after sampling, with adequate information to ensure that the specimen is traceable to the client and the accompanying request form.
A minimum of two identifiers shall be used to label the sample tube/container. This shall include but not limited to the following:

- Client full name*
- Unique identifier*
- Age and Sex*
- Specimen and test requested*
- Date and Time of Sample Collection
- Initials of person collecting sample

*Shall be included in tiny tubes/containers

4.1.7 Specimen packaging and storage

Specimen packaging and storage should be made in accordance with the instructions set out in this guideline or related SOP. For transport purposes, the triple packaging system shall be used as follows:

- **Primary receptacle**

This is a primary watertight, leak-proof and appropriate receptacle containing the samples. The receptacle is packaged with enough absorbent material to absorb all fluid in case of leakage and/or breakage.

- **Secondary packaging**

A secondary durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to

absorb all fluid in case of breakages. Secondary receptacles should not be overloaded with too many primary receptacles.

•• **Outer packaging**

Secondary packaging is placed in outer shipping packaging with suitable cushioned material. For specimen that require cold temperatures for transport, ice packs should be placed in the outer container. Outer packaging shall be designed to protect their contents from outside influences, such as physical damages, while in transit. The outer packaging shall be appropriately labeled with the following:

a. Package markings (e.g. biohazard symbol)
b. Proper shipping name
c. 'To' and 'From' labels

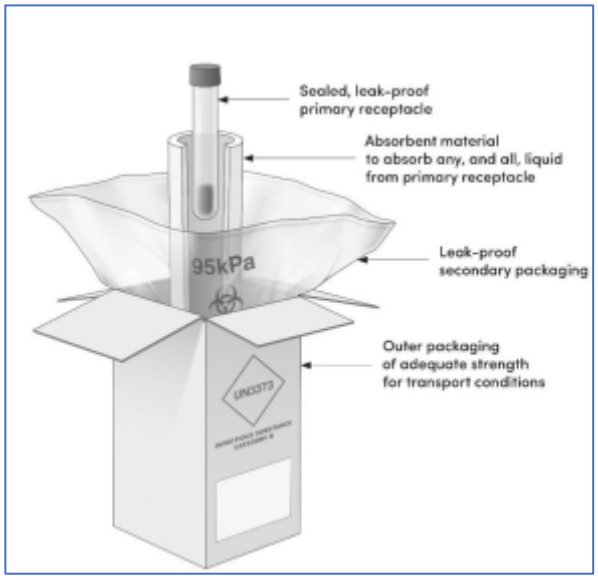

Figure 1: Triple packaging[2]

4.1.8 Specimen storage

Samples should be transported according to the arrangements set out in this guideline and according to relevant SOPs.

The principle of safe transport is that the material should not have any possibility of escaping from the package under normal conditions of transport.

The following practices should be observed:

•• Sample containers should be watertight and leak-proof;
•• If the sample container is a tube, it must be tightly capped and placed in a rack to maintain it in an upright position;
•• Sample containers and racks should be placed in robust, leak-proof plastic or metal transport boxes with secure, tight-fitting covers;
•• The transport box should be secured in the transport vehicle;
•• Each transport box should be labelled appropriately consistent with its contents;
•• Sample data forms and identification data should accompany each transport box;
•• A spill kit containing absorbent material, an appropriate disinfectant (e.g. 10% chlorine), a leak-proof waste disposal container and heavy-duty reusable gloves should be kept in the transport vehicle.

[2] WHO, Guidance on regulations for the transportation of infectious substances – 2021-2022

- Personnel trained on the safety handling of specimens should be engaged. Trainings should be routinely organized for such personnel.
- All samples should be transported under appropriate temperatures maintaining specimen integrity
- Each specimen should be accompanied with a duly filled request form and a specimen transport log
- Specimen should be transported in a manner that ensures the safety of the carrier, the public and the receiving laboratory in compliance with established requirement.

NB: In case of any unlikely events (such as accident), the transporting officer shall take steps to contain the spread of the spillage (where possible) and alert the referring and receiving facility for appropriate intervention.

4.1.9 Specimen acceptance and rejection criteria

Each laboratory shall define samples quality requirements and rejection criteria. The rejection criteria should be communicated to all laboratory users.
The following are examples of reasons for specimen's rejection:

- Incomplete request forms
- Request form not included with the specimen
- Specimen identification is missing or incorrect or does not correlate with the information on request form
- Unlabeled or improperly labeled specimen
- Specimens received in leaking, cracked or broken containers
- Specimens with obvious (visually, apparent) contamination
- Expired tubes or other collection device
- Incorrect temperature and/or packaging of specimen
- Stability of the analyte in the specimen has been exceeded (e.g. specimen is too old upon receipt)
- Inadequate volume or overfilling of specimen container
- Incorrect specimen container or tube
- Specimen not included with the request form
- Specimen hemolyzed or clotted

Exceptions to rejection of samples may be made for critical samples that may be difficult or impossible to replace e.g. CSF, tissue etc. In case of sample rejection, the laboratory should inform the concerned personnel and arrange for a recollection.

The final report for such critical samples shall indicate the nature of the problem and interpretation of the results should be done in caution.

4.2 Specimens collection procedures

4.2.1.1 Blood specimens

4.2.1.2 Venous blood

Order of Draw

Collect blood specimens in the order described in the table below:

Table 1 Example of order of draw of blood samples

#	Type of tube/usual colour	Additive	Tests/uses	Special instructions
1	Blood culture bottle	Broth mixture	Microbiology – aerobes, anaerobes, fungi	Volume stated on bottle, Gently invert 8 – 10 times after sampling
2	Blue top tube	Sodium citrate	Coagulation tests (prothrombin time, fibrinogen & D-dimer)	Full draw required (Volume stated on tube, invert gently 3 – 4 times
3	Red top tube with clot activator	Clot activator, silicon coated (plastic)	Most clinical chemistry, serology, immunology & toxicology	Cannot be used for CSF specimens Gently invert 5 - 6 times after sampling
4	Yellow, red-grey tiger top or gold	None, clot activator & gel	Most clinical chemistry, serology, immunology & toxicology	Gently invert 5 – 6 times after sampling to ensure mixing of clot activator & blood
5	Green top	Sodium or lithium heparin (light green with gel)	Certain clinical chemistry tests e.g. Troponin T & ammonia, genetic studies & flow cytometry	For lithium level use sodium heparin, for ammonia level use either
6	Purple/Lavender Top tube	K_2EDTA	Hematology, Blood Bank (crossmatch), Viral load	Gently invert 8 - 10 times after sampling to ensure mixing of anticoagulant with blood to prevent clotting
7	Black top tube	Sodium citrate (buffered)	Erythrocyte Sedimentation Rate (ESR)	Full draw required Gently invert 8 – 10 times after sampling to ensure mixing of additives with blood
8	Pale yellow top tube	Acid-citrate-dextrose (ACD, ACDA or ACDB)	HLA tissue typing, paternity testing, DNA studies	
9	Grey Top tube	Sodium fluoride & potassium oxalate or Na_2EDTA	Glucose & lactate	Full draw required (may cause hemolysis if short draw) invert 8 times after sampling to ensure mixing of additives with blood

PLEASE NOTE that the color of the collection tube top may vary slightly between different manufacturers. Please select collection tubes according to the desired additive.

The following sites should be avoided for sample collection:

- Areas with extensive scarring from burns or surgery
- The upper extremity on the side that a mastectomy was performed
- Hematoma – A venipuncture should not be performed on a hematoma, regardless of how small it may be. If there is not an alternate vein to draw, the venipuncture should be performed distal (below) to the hematoma
- Avoid multiple venipuncture from same site
- Intravenous therapy/Blood Transfusions – If it is not possible to draw the opposite arm, then blood should be drawn distal to the intra venous line.

Procedure for venipuncture

General

Appropriate venipuncture site selection is important for efficient phlebotomy. Assess the arm for visibly accessible veins. The median cubital vein in the antecubital fossa is the preferred venipuncture site. Although the larger and fuller median cubital and cephalic veins of the arm are used most frequently, wrist and hand veins are also acceptable for venipuncture.

Specific material and supplies:

- Dacron /Rayon swab
- utility cart
- blood collection trays
- gloves
- blood collection system with safety features (needles and holders, or needles with integrated holders)
- blood collection tubes (a full range of tubes with different volume, within the expiry date)
- tourniquet (where appropriate, single use)
- antiseptics to clean the puncture site
- bandages/Phlebotomy plaster
- cotton wool/gauze pads
- sharps bin
- leak proof transportation bags

Procedure for venipuncture

- Approach the patient in a friendly, calm manner. Provide for their comfort as much as possible and gain the patient's co-operation.
- Verify the patient's condition. Fasting, dietary restrictions, medications, timing, and medical treatment are all of concern and should be noted on the lab request form.
- Position the patient. The patient should sit in a chair, lie down or sit up in bed.
- Carefully hyperextend the patient's arm
- Apply the tourniquet 7.5-10 cm above the selected puncture site. Do not place too tightly or leave on for more than 2 minutes.
- The patient should make a fist (so the veins are more prominent) without pumping the hand. Select the venipuncture site.
- Prepare the patient's arm using an alcohol prep. Cleanse in a circular fashion, beginning at the site and working outward. Allow to air dry.
- Grasp the patient's arm firmly using your thumb to draw the skin taut and anchor the vein. The needle should form a 15 to 30-degree angle with the surface of the arm. Swiftly insert

the needle through the skin and into the lumen of the vein. Avoid excessive trauma and probing.
- When the last tube to be drawn is filling, remove the tourniquet.
- Remove the needle from the patient's arm using a swift backward motion.
- Press down on the gauze once the needle is out of the arm, applying adequate pressure to avoid the formation of a hematoma.
- Dispose of needle in the sharp's container WITHOUT RECAPPING.
- Dispose of contaminated materials/supplies in the designated waste containers.
- Have the patient hold a small gauze pad over the puncture site for a couple of minutes to stop the bleeding. Cover the puncture site with sterile gauze, held in place with an elastic plaster.
- Mix and label all appropriate tubes at the patient bedside. Label the tubes with the appropriate patient information as described.

4.2.1.3 Capillary blood

General

Capillary blood is mainly collected when the patient is an infant or young child and/or the volume of blood required is small, e.g. Packed Cell Volume (PCV) measurement and to make thick and thin blood films. Thick films for malaria parasites are best made from capillary blood (anticoagulated blood is more easily washed from slides during staining).
Capillary blood can be collected from the 'ring' finger of a child or adult or the heel or toe of an infant.

Specific material and supplies:
- Lancet
- Microcontainer tubes
- Gauze
- Alcohol prep
- Sterile bandage

Procedure
- Position the patient so that the hand is easily accessible
- Cleanse the fingertip of the 3rd (middle) or 4th (ring) finger with an alcohol prep
- Using a sterile lancet, puncture the fingertip in the fleshy part of the finger, slightly to the side.
- Wipe away the first drop of blood with a sterile gauze.
- Gently massage finger to maintain blood flow
- Cap sample collection tube and mix appropriately
- Apply sterile adhesive bandage over the puncture site

The best positions for fingerprick and heelprick are shown in Figures 2 below

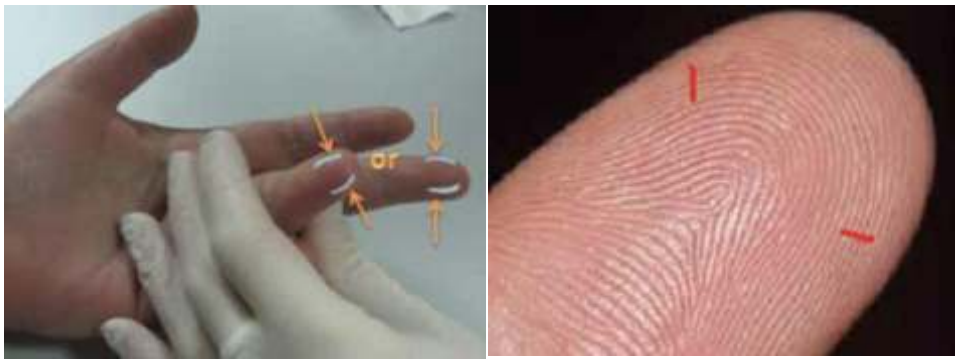

Figure 2: Site selection for fingerprick[3]

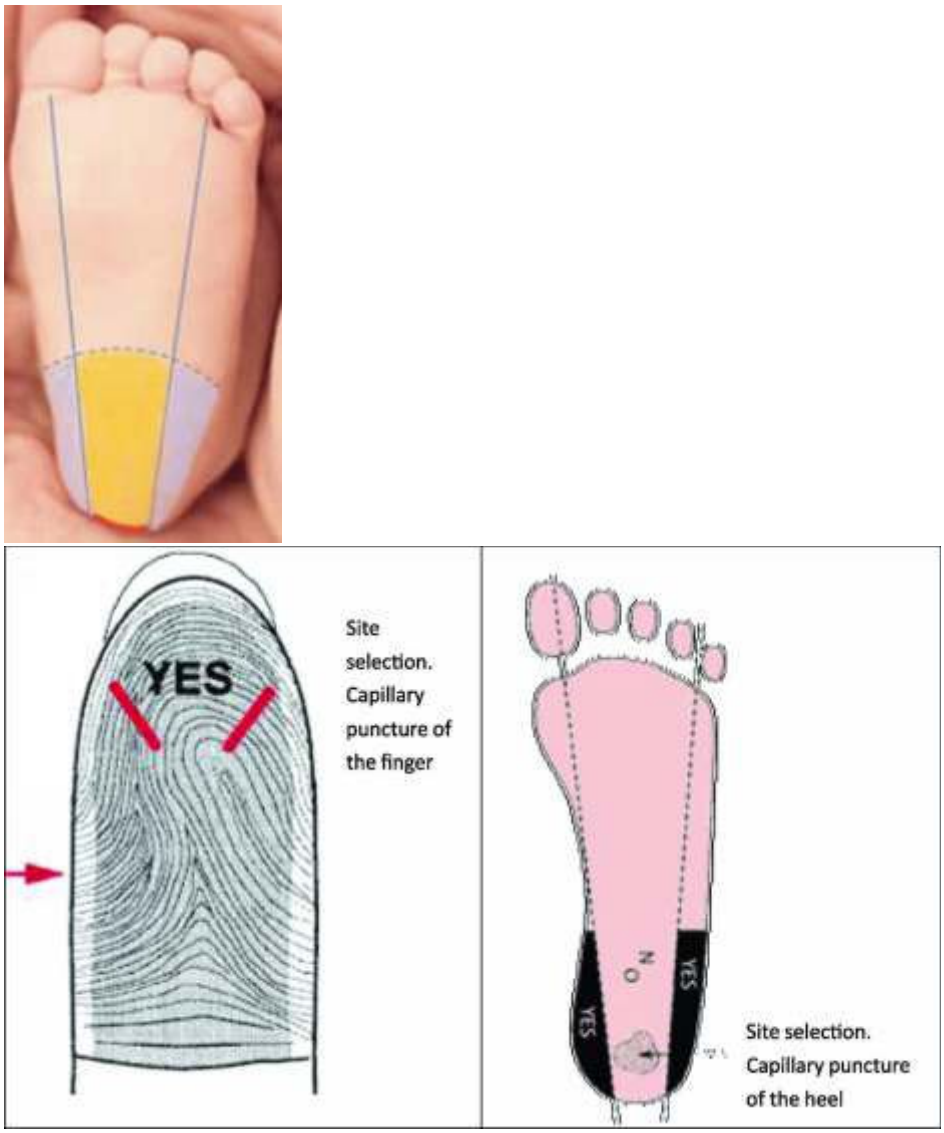

Figure 3: Site selection for heelprick[4]

[3] Krleza, Jasna Lenicek et al (2015) "Capillary blood sampling: national recommendations on behalf of the Croatian Society of Medical Biochemistry and Laboratory Medicine." *Biochemia medica* vol. 25,3 335-58. doi:10.11613/BM.2015.034

[4] Krleza, Jasna Lenicek et al (2015) "Capillary blood sampling: national recommendations on behalf of the Croatian Society of Medical Biochemistry and Laboratory Medicine." *Biochemia medica* vol. 25,3 335-58. doi:10.11613/BM.2015.034

4.2.1.4 Dried blood spot (DBS)

General

Dried blood spot specimens are collected by applying a few drops of blood, drawn by lancet from the finger, heel or toe, onto specially manufactured absorbent filter paper. The blood is allowed to thoroughly saturate the paper and is air dried for at least 4 hours.

Specific material and supplies:

- Filter cards
- Pipette

Preparation from blood collected by venipuncture

- Spot the collected anti-coagulated (EDTA) whole venous blood on the filter cards as soon as possible. Do not prepare dried blood spots more than 24 hr. after venipuncture.
- Put all the information necessary for the identification of the patient on the filter card. One card should be spotted only with the blood of a single individual.
- Put on disposable latex rubber gloves.
- Gently invert the blood collection tube 2 – 4 times and subsequently open the stopper carefully.
- Aspirate 50 µl of whole venous blood using a pipette with a disposable tip. Transfer the blood to the center of one circle without touching the filter paper directly with the tip of the pipette. Try to fully saturate the circle.
- Repeat this procedure to fill all required circles of the card.

Preparation from blood collected by skin puncture

1. Wipe off the first drop of blood with a gauze pad because it may contain excess tissue fluids. Massage the finger again to increase blood flow at the puncture site. Transfer the following drop to one of the circles of a filter card without touching the surface directly with the fingertip. Allow the blood to be soaked into the texture of the filter by capillary forces only.
2. Let the next large drop of capillary blood form on the fingertip and collect it in the next circle. Continue this procedure until all necessary circles are filled or blood flow stops.
3. Do not squeeze or “milk” the finger excessively if the blood flow is not sufficient to fill all the required circles of the filter card. If blood flow stops, place a bandage/cotton wool on the fingertip. Perform a second skin puncture on another finger if more blood is needed for the examination.

Drying of Blood Spots

- To dry the blood spots, put the filter cards on a clean paper towel in a biohazard safety cabinet and let them dry, preferably O/N (but for at least 4 h), at room temperature in the absence of any external source of heat.
- When the drying process is complete, the blood spots have a uniformly dark brownish color and no red areas are visible anymore (See figure B)

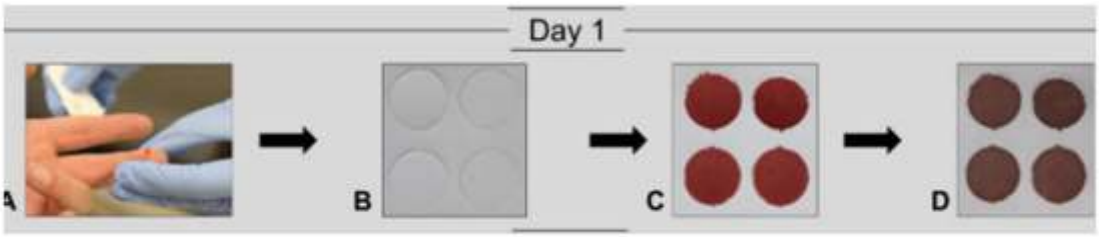

Figure 4: DBS drying process[5]

4.2.1.5 Blood cultures

General

Blood cultures are performed on suspicion of an infection in the blood (sepsis)

Procedure for site selection and preparation

Select a different site for each blood sample
Avoid drawing blood through indwelling intravenous or intra-arterial catheters
Vigorously cleanse the venipuncture site with 70% isopropyl or ethyl alcohol (chlorhexidine may also be used)
Starting at the center of the selected site, swab in a circular motion outward (concentrically) with 1 to 2 % tincture of iodine for 30 seconds or with 10% povidone-iodine solution for 1 minute. If these solutions are not available, 70% isopropyl alcohol can be used instead
Allow alcohol/povidone iodine solution at the venipuncture site to air-dry
Remove the lid without touching the top of the blood culture bottle. If contamination occurs during removal of the lid, alcohol can be used to disinfect the top.

Note 1: Do not touch the venipuncture site after preparation and prior to phlebotomy.

Note 2: Do not use iodine on the tops of the BacT/Alert bottle

Collection of blood

Using syringe and needle, insert the needle into the vein and draw blood. Do not change needles before injecting the blood into the culture bottle. The closed system consisting of a vacuum bottle and double-needle collection tube can be used.

- After the blood is inserted into the blood culture system, mix gently and well to avoid clotting
- Use a new needle if the vein is missed initially
- Add sufficient volume of blood to attain the appropriate ratio of blood to medium
- After phlebotomy, apply pressure with piece of gauze or cotton wool to stop the bleeding. Cleanse the site with 70% alcohol to remove remaining iodine that can cause irritation in some patients and cover puncture wound appropriately

Specimen volume

The volume of blood is critical because the number of organisms in the majority of bacteremia is low, especially if the patient is on antimicrobial therapy.

[5] Grüner N, Stambouli O, Ross RS(2015). Dried blood spots--preparing and processing for use in immunoassays and in molecular techniques. *J Vis Exp*. 2015;(97):52619. Published 2015 Mar 13. doi:10.3791/52619

- **Children:** Infants and children have a lower total blood volume. 1 to 5 ml of blood should be drawn per venipuncture; a minimum requirement is 0.5ml per blood culture.
- **Adults:** 20 ml blood distributed between two aerobic bottles. If clinical diagnosis indicates an anaerobic infection the initial volume drawn should be 20 ml of blood, which is divided equally between the aerobic and anaerobic bottles.

4.2.2 Respiratory Tract Specimens

4.2.2.1 Throat swab

General

A throat swab is taken to support the detection of the presence of group A streptococcus bacteria, the most common cause of strep throat. These bacteria also can cause other infections, including scarlet fever, abscesses, and pneumonia.

Specific material and supplies:

- Dacron /Rayon swab
- Appropriate PPE (Laboratory coats, gowns, aprons, Gloves, Face mask, face shield, etc.)
- Tongue depressor
- Bright light source
- Appropriate transport media

Procedure

- Rinse the mouth with clean water to remove food and other particles where necessary
- Shine a bright light into the oral cavity of the patient so that the swab can be guided to the posterior pharynx (if required).
- The patient is instructed to tilt his/her head back and breathe in deeply.
- Depress the tongue with a tongue depressor to help visualize the posterior pharynx.
- Use a sterile Dacron/Rayon swab. Extend the swab to the back of the throat between the tonsil pillars and behind the uvula.
- Have the patient phonate a long 'aah' which will lift the uvula and help to prevent gagging.
- The tonsil areas and posterior pharynx should be firmly rubbed with the swab.
- Care should be taken not to touch the teeth, cheeks, gums or tongue when inserting or removing the swab to minimize contamination with normal mouth flora.

4.2.2.2 Nasal swabs

General

Submitted primarily for the detection of nasal carries of *Staphylococcus aureus*.

Specific material and supplies:

- Dacron /Rayon swab
- Appropriate PPE (Laboratory coats, gowns, aprons, Gloves, Face mask, face shield, etc.)
- Bright light source
- Appropriate transport media

Procedure for nasal swab collection:

- Insert a moistened sterile swab into the nose until resistance is met at a level of the turbinate
- Rotate the swab against the nasal mucosa
- Repeat the process on the other side using the same swab

- Place in suitable transport media and send to the laboratory within 2 hours

NB: If sample is not transported immediately, store at a required temperature.

4.2.2.3 Nasopharyngeal swabs

General

For the diagnosis of COVID-19 and other respiratory infections, nasopharyngeal sampling is preferred. Oropharyngeal sampling alone is done if for any reason nasopharyngeal sampling is not possible. In the case of a combined oropharyngeal and nasopharyngeal swab, always start with the oropharyngeal swab.

Specific material and supplies:

- Calcium alginate-tipped swab/Dacron /Rayon swab
- Appropriate PPE (Laboratory coats, gowns, aprons, Gloves, Face mask, face shield, etc.)
- Tongue depressor (where applicable)
- Viral transport media

Procedure

Refer to the national SOP for COVID-19 specimen collection

4.2.2.4 Oropharyngeal swab

Specific material and supplies:

- Dacron /Rayon swab
- Appropriate PPE (Laboratory coats, gowns, aprons, Gloves, Face mask, face shield, etc.)
- Bright light source
- Appropriate transport media

Procedure:

- Swab the posterior pharynx, tonsils, and other inflamed areas. Avoid touching the tongue, cheeks, and teeth with the swab when collecting specimens.
- Remove and place the swab into the viral transport medium. Break swab at the indicated break line and cap the specimen collection tube tightly

4.2.2.5 Nasopharyngeal/Nasal Aspirate

Specific material and supplies

- Sterile suction catheter/suction apparatus
- Transport media (where applicable)
- Specimen container
- Appropriate PPE (Laboratory coats, gowns, aprons, Gloves, Face mask, face shield, etc.).

Procedure:

- Attach catheter to suction apparatus
- Tilt patient's head back 70 degrees
- Insert catheter into nostril (catheter should reach depth equal to distance from nostrils to outer opening of ear)
- Begin gentle suction. Remove catheter while rotating it gently
- Place specimen in a sterile container (transport media where applicable).

4.2.2.6 Bronchoalveolar lavage (BAL)

General

Done only by trained professionals

Specific material and supplies

- Sterile wide-mouthed, unbreakable, leakproof, screw-capped container

Procedure:

- For Microbiology, collect the specimen in a sterile, labeled container. Do not add preservative.
- For Cytology, the specimen may be collected in a sterile container with or without preservative

4.2.2.7 Tracheal aspirates

General

Done only by trained professionals

Specific material and supplies

- Sterile wide-mouthed, unbreakable, leakproof, screw-capped container

Procedure

- For Microbiology, collect the specimen in a sterile, labeled container. Do not add preservative.
- For Cytology, the specimen may be collected in a sterile container with or without preservative

4.2.3 Sputum

General

Sputum specimen is collected by the patient. The laboratory should provide clear instructions to the patient:

Specific material and supplies

- Clean screw-cap universal containers: Wide-mouthed, unbreakable, leakproof, screw-capped containers. Containers should have a volume capacity of 50 ml and made of translucent material in order to observe specimen volume and quality without opening the container.

Procedure

- Sputum should be collected in open air or in a well-ventilated area preferably early morning. However, sample may be collected at any time sputum is available to be produced.
- Give each patient a new clean sputum container. If two samples are required, give two separate containers. Sputum containers or mugs should never be re-used.
- Give the patient instructions and demonstrate how he or she can produce and collect good sputum.
- Instruct patient to gargle or rinse mouth with clean water.
- Ask patient to do up to three deep inhalations and exhalations and on the third exhalation or when a strong cough reflex arises, accompany it with a cough from deep inside the chest as much as possible (do demonstrative actions for the patient).

- Demonstrate to the patient how to open and close the sputum container so as there are no leaks or smearing on the exterior of the container.
- Emphasize the need for the patient to supply the most useful specimen, the normally thick, yellowish (sometimes blood-stained), purulent material brought up from the lungs after a deep, productive cough.
- Emphasize that saliva produced by spitting is not sputum. However, if the only sample the patient can produce is salivary, do submit it to the laboratory as it can still yield useful information.
- Encourage the patient to bring the collected specimen back to the unit as quickly as possible.
- Fill in all details on the request form/case base form and submit along with the specimen to the laboratory.

4.2.4 Eye swab

General

Culture of Eye swabs for the isolation and identification of bacterial & fungal pathogens

Specific material and supplies

- Calcium alginate swabs
- Dacron or cotton wool swabs
- Transport medium

Procedure:

- Sample each eye with a separate swab.
- Pre-moisturize swab with sterile saline.
- Carefully roll swab over each conjunctiva.
- Inoculate directly onto culture plates, if possible, or break tips into appropriate medium.
- For chlamydial cultures use calcium alginate swabs.
- For viral cultures use dacron or cotton wool swabs with non-wood shafts.

4.2.5 Ear swab and middle ear effusion

Specific material and supplies

- Dacron or cotton wool swabs
- Appropriate transport medium
- Sterile saline

Procedure

- Swab any pus or exudates
- For investigation of fungal infection, scrapings of material from the ear canal are preferred although swabs can also be used
- Collect specimens other than swabs into appropriate leakproof containers

For outer Ear:

- Remove any debris or crust from outer ear
- Moisten swab with sterile saline
- Obtain sample by firmly rotating swab in outer canal
- Break tip in an appropriate transport medium

4.2.6 Urogenital specimen

General

Vaginal, cervical and endocervical swabs (preferably taken by a female worker otherwise in the presence of a female attendant).

- The patient must refrain from any kind of sexual activity, douching, and inserting any intravaginal products for at least 48 hours prior to the collection of vaginal/cervical specimens
- Place the patient in the gynecological position;
- Clean the vulva with an antiseptic swab from top to bottom and rinse with a saline water swab, always from top to bottom;
- Place the speculum and illuminate the cervix and vagina.

Specific material and supplies

- Disposable speculum
- Swabs, dacron polyester (sterile, individually wrapped)
- Plastic pear
- Transport medium
- Bright light

Procedure for cervical swab

- Clear away vaginal mucus and exudates
- Insert and open the speculum to expose the cervix
- Insert a swab in the cervix so as to obtain the exudates. Break off the tip into the transport medium

Procedure for high vaginal swab

- Clear away excess vaginal mucus and exudates using a swab
- Insert a dacron swab (dipped in sterile saline water) and gently rotate at 360 degrees in all four quadrants of the vaginal vault and break tip into appropriate transport medium.

Procedure for endocervical swab

- Remove any excess mucus or vaginal secretions
- Insert and open the speculum to expose the cervix
- Insert the swab into the cervix and leave it inside for a few seconds and gently remove to avoid contact with the vaginal mucosa

4.2.6.1 Urethral Swab

Specific material and supplies

- Appropriate swabs (dacron, alginate)
- Appropriate transport media (E.g. swab with 2SP transport media for Chlamydia and Mycoplasma)

Procedure for female urogenital sample collection:

- Massage urethra against pubic symphysis through the vagina and collect specimen with sterile swab; break tip into appropriate transport medium

Procedure for male urogenital sample collection:

- Disinfect the glans penis with appropriate disinfectant (preferably 70% ethanol).

- Express urethral exudate onto swab from distal urethra.
- If there is no exudate, collect 1 hour after urination. Clean glans penis with soap under running water.
- Using aseptic technique, insert urogenital swab 2 – 4 cm into the urethra, rotate swab and leave in place for 2 seconds.
- Remove and break tip into appropriate transport medium.

4.2.6.2 Procedure for prostatic fluid/Swab

- Clean the glans penis with soap under running water after the patient has urinated.
- Massage prostate through the rectum
- Collect sample directly into sterile plain tube or take a swab where necessary

4.2.6.3 Semen specimen

General

- Semen specimen should be collected between two and seven days from the time of most recent ejaculation.
- Specimen must be delivered to the laboratory within one hour of collection, which is most easily accomplished if it is collected in the laboratory.
- Specimen can be collected at home provided that it can be delivered to the hospital within one hour.

Specific material and supplies

Appropriate container provided by the laboratory

Procedure for semen sample collection and transport

- Collect the entire specimen by masturbation using the supplied container
- Do not use lubricants or other substances that may contaminate the specimen
- Inform lab staff if part of the specimen is not collected
- Do not use a condom (spermicidal condoms) to collect the specimen; condoms can kill or damage sperm cells, and it is impossible to get the entire sample out of a condom. However, specialized non-toxic condoms for semen collection can be used
- Take note of the time the specimen is collected. That time will need to be indicated on the request form in the laboratory
- The specimen must be kept at room or body temperature (20 - 37°C) while being transported

4.2.7 Pus specimens & wound discharge

Specific material and supplies:

- Sterile saline
- Appropriate PPEs
- Syringe and needle
- Cotton or dacron swab
- Specimen transport bag
- Sterile container

Procedure for collecting closed wound/ abscess sample

- Remove surface exudate by wiping with sterile saline
- Allow surface to dry
- Using a needle with syringe, aspirate abscess wall material
- Remove needle using a protective device
- Transfer the aspirated material into a sterile container. Also inoculate anaerobic transport if anaerobic infection is suspected.
- Deliver promptly to the Microbiology laboratory.

Procedure for collecting open wound samples

- Remove surface exudate by wiping with sterile saline.
- Allow surface to dry.
- If possible, aspirate.
- Alternatively, pass a swab(s) deep into the lesion and firmly sample the lesion's advancing edge. For mycobacterial culture, 2 swabs are preferred. (NB: If anaerobic bacteria is suspected, take a swab deep into the wound).
- Return swab(s) to transport sleeve.
- Label appropriately
- Deliver promptly to the Microbiology laboratory.

4.2.8 Body Fluids (pericardial, ascetic, pleural, peritoneal, synovial and amniotic fluids)

Specific material and supplies

- Syringe
- Blood culture bottle
- EDTA tube
- Universal container

Procedure:

- Clean percutaneous aspiration site with appropriate disinfectant
- Aseptically perform percutaneous aspiration with syringe and needle to obtain at least 3-5 ml fluid and transfer to appropriate containers
- Transport immediately to Lab.
- Always submit as much fluid as possible.

4.2.9 Cerebrospinal fluid

General

Lumbar punctures (LPs) are performed to collect cerebrospinal fluid (CSF) for laboratory evaluation to establish a diagnosis of infection (e.g. bacterial, fungal, mycobacterial, or amebic meningitis), malignancy, subarachnoid hemorrhage, multiple sclerosis, or demyelinating disorders. CSF has to be collected into three tubes, which do not contain any anticoagulant. The tubes are distributed to the appropriate laboratory according to their sequence of collection. The following description indicates the specimen conditions and suitability of the sample for each test type.

- First tube is for clinical chemistry for chemical analysis.
- Second tube is suitable for microbiological testing.
- Third tube is useful for cell counts.
- If only one tube of CSF is collected, it should be submitted to the microbiology laboratory first.

Material and supplies:

- Surgical mask
- Appropriate PPEs
- Appropriate tubes
- Apparatus for lumbar puncture
- Antiseptic (70% alcohol; povidone iodine)

Procedure:

- CSF collection should be done only by trained professionals. Lumbar puncture is an invasive procedure laboratorian should not attempt it.
- The specimen should be transported to the laboratory promptly and processed as soon as possible.
- Transport CSF to the laboratory under appropriate temperatures:
  - ❖ CSF for Chemical Analysis: 2 – 8°C
  - ❖ CSF for Microbiology testing: Room temperature (24-30°C)
  - ❖ CSF for Cell Count: 2 – 8°C

NB: For long-term storage prior to transportation, store CSF samples at -20°C or below.

4.2.10 Tissues and biopsies

Materials and Supplies

- Appropriate PPE
- Sterile container
- Appropriate preservative (e.g. 10% buffered Formalin)
- Sterile saline

Procedure:

- Tissue collection is an invasive procedure. Collected only by a trained professional
- Collect tissue aseptically including material both from the center and edge of the
- lesion.
- Place the specimen in a sterile container on sterile gauze moistened with sterile **non-**
- bacteriostatic saline.
- Transport in less than an hour at ambient temperature. For virology cultures,
- transport in viral transport media.
- Do not submit tissue in formalin (for microbiological cultures).

4.2.11 Dermatological specimens including skin scrapings, hair & nails

Specific material and supplies

- 70% Alcohol
- Scalpel
- Nail clippers
- Forceps
- Sterile container

Procedure:

- Cleaned area with 70% alcohol
- Skin sample should be scrapped from the margin of the lesion using a scalpel and placed on a clean dry paper

- Affected nail should be scraped with a scalpel to remove the outermost layer
- Place the specimen in a dry, sterile container. Clippings from discolored part of the nail are acceptable
- Collect at least 10-15 strands of hair from the root using forceps. **Cut hair is not an acceptable specimen.**

4.2.12 Urine

General

- Urine is normally a sterile body fluid. If specimens are not collected adequately, contamination by normal flora of the perineum, urethra and vagina can occur.
- Early morning and mid-stream urines are the preferred specimens (for C/S) and have the best yield. For routine examination, samples can be collected at any time of the day.

Specific material and supplies

- Sterile container
- Sterile Syringe
- 70% Alcohol

Procedure:

- Both males and females should pass a few milliliters of urine into the toilet bowl, DO NOT STOP THE FLOW, and then collect the midstream portion of urine into a sterile container. Place the lid on the cup securely.
- Transport specimen to the laboratory within one hour for bacteriological examination, because of the continuous growth of bacteria in vitro thus altering the actual concentration of organisms. In case of delay anticipated for more than two hours, refrigerate the sample at 4-6 degrees.
- Reject specimen >48 hours unless they were appropriately refrigerated
- In case of suspected urethritis, first part of the voided urine is collected. In case of urine sample for suspected TB approximate 40 mL of early morning sample for 3 consecutive days should be collected.

> **NOTE:** Do not take in fluids to induce urine production prior to collection as this will dilute colony counts and result in potential misinterpretation.
> **Never submit urine collected in a bedpan or urinal.**

Sample collection from Indwelling catheters:

- Do not collect urine from the drainage bag because growth of bacteria outside the catheter may have occurred at this site.
- Clean the catheter with an alcohol pad.
- Use a sterile needle and syringe to puncture the tubing. Aspirate the urine directly from the tubing.
- Transfer the urine to a sterile specimen container or appropriate transport media.
- Urine catheter tip cultures are not acceptable.

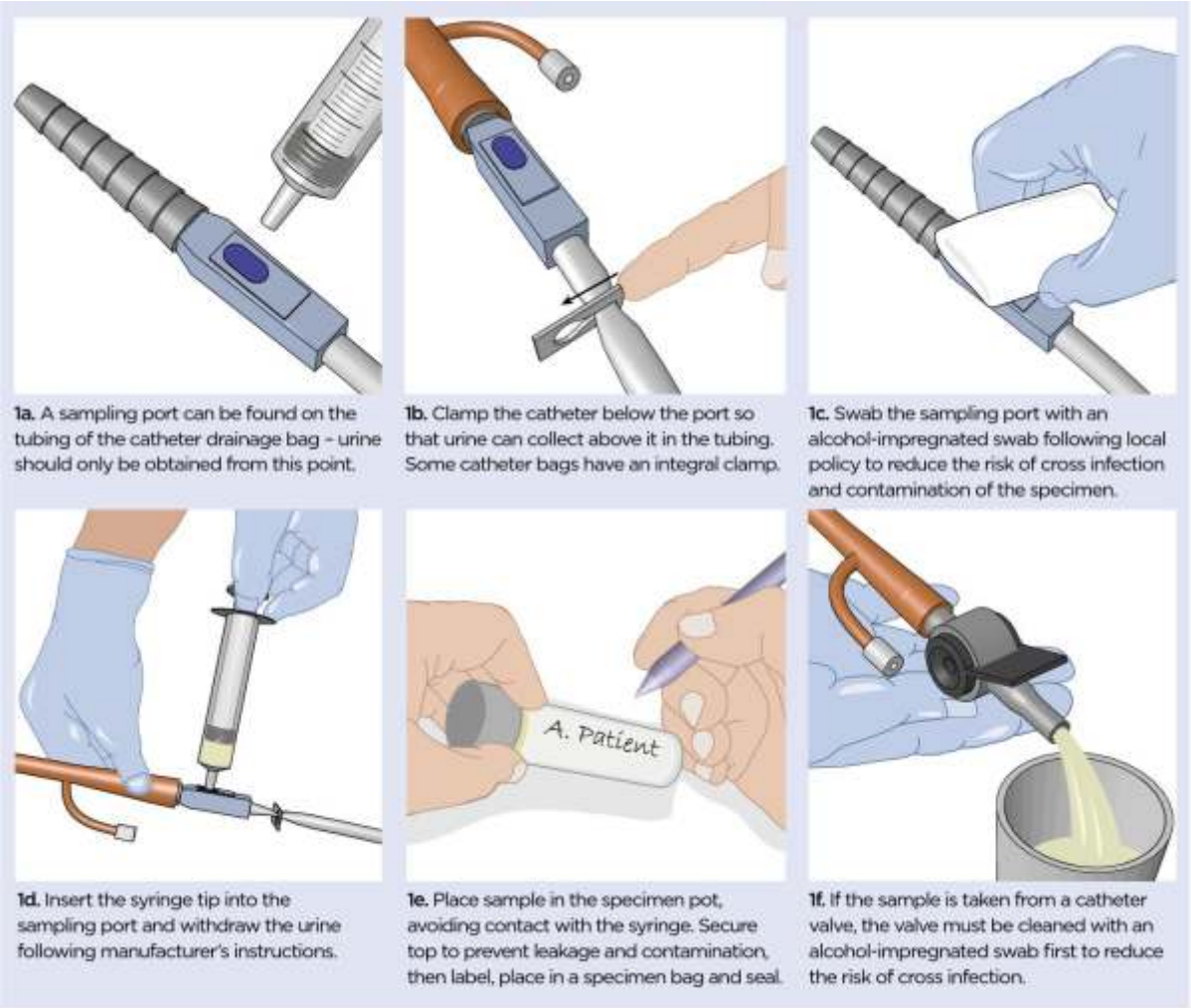

Figure 4 Procedure to collect urine from indwelling catheters[6]

[6] **Shepherd E** (2017) Specimen collection 1: general principles and procedure for obtaining a midstream urine specimen. *Nursing Times*; 113; 7, 45-47.

Suprapubic aspirate (SPA)
General
Done only by trained professionals

Specific material and supplies
- Syringe 3mL or 5mL
- Sterile gloves
- Specimen jar

Procedure

- Hold the child securely held in supine position with legs extended and together or in a frog leg posture (clinician preference).
- Skin prep: cleanse a wide area of the lower abdomen with the recommended antiseptic solution. Allow skin to dry prior to needle insertion
- Identify the needle insertion point: abdominal midline, 1-2 cm above the pubic symphysis, along the lower abdominal crease. The aim is for the needle to puncture the centre of a full bladder.
- Insert the needle perpendicular to the skin in all directions. Anatomically, this equates to advancing the needle through the abdominal wall in a slightly cephalad/umbilical direction, angled approximately 20degrees from the vertical, while applying mild negative pressure to the syringe.
- Do not aim the tip of the needle down into the pelvic region as the bladder is predominantly abdominal in infants.
- Puncture the skin quickly, as if popping a balloon
- Advance 2-3 cm, to the hub of the needle if needed, and aspirate. A distinct change in resistance may be felt as the needle passes through the bladder wall.
- If urine is not immediately aspirated, continue aspirating as the needle is withdrawn
- If unsuccessful, withdraw the needle to just under the skin and advance at an angle with the needle aimed slightly more cephalad (towards the umbilicus, away from the pelvis). Do not repeat this procedure
- If still unsuccessful, further attempts should be at the discretion of the senior medical officer and an alternate method of urine collection should be considered. The child should be hydrated in the interim.
- When urine is obtained, remove the needle from the syringe prior to expelling the sample into a sterile labelled container. Seal the specimen jar in a transport bag with the completed request form, at the bedside. A urinalysis should be performed on part of the sample prior to transport to the laboratory.
- An adhesive dressing may be applied to the puncture site

Notes:
a. Urine for CMV culture must be received within 1 hour of collection. b. Minimum urine volume for AFB culture is 40 ml.

4.2.13 Stool

Specific material and supplies

- Sterile container
- Rectal swabs
- Appropriate transport medium (e.g. Cary Blair medium)
- Appropriate preservative (10% formalin)
- Modified Polyvinyl Alcohol (PVA)

Procedure:

- Specimens should be collected in sterile leak-proof containers. Universal containers are suitable provided lids are closed properly.
- Containers should be clean and dry. The presence of water or urine can result in inaccurate interpretation.
- Transfer specimen into an appropriate transport medium (e.g. Cary-Blair) container for routine bacterial stool culture.
- For *C. difficile* and fecal lactoferrin testing, place stool in sterile container. For ova and parasite (O&P), use 10% formalin and modified PVA. Transport at ambient temperature within two hours of collection and store at 2-8 °C.
- Only loose or diarrheal stools are recommended for routine bacterial and *C. difficile* cultures and PCR.

If a stool specimen is not available, the following are suitable alternatives for culture:

- A swab of rectal mucus, or
- A rectal swab inserted one inch into the anal canal,
- Immediately insert the swab into the transport medium and deliver to laboratory promptly. If delays are anticipated, the swab in transport medium can be refrigerated.

5 Animal specimens

5.1 General animal specimen's collection and management guidelines

Laboratory testing of animal disease is critically dependent on the quality and appropriateness of the samples collected for analysis. Consequently, communication between the practitioner and the veterinary diagnostic laboratory is critical to ensure that the appropriate test and sample are used and that results are interpreted correctly in order to provide optimal service to the client. The quality of the samples and the information accompanying the samples greatly facilitate the ability to pick the best test and get reliable results that will be useful for disease diagnostic and surveillance. The samples must be shipped to the laboratory under conditions that are appropriate for subsequent processing and testing.

This section is describing general consideration for sample collection, shipment and storage of samples collected from animals. Veterinary laboratory should be contacted to obtain information and recommendations needed for specific details on sampling requirements (type, preservatives, handling procedures etc.). Also, laboratory staff can provide guidance when there are additional questions as well as helping in interpretation of test results.

Additionally, all specimens must be collected using appropriate biosafety and containment measures in order to prevent contamination of the environment, animal handlers, and individuals doing the sampling as well as to prevent cross-contamination of the specimens themselves. Care should additionally be taken to avoid undue stress or injury to the animal and physical danger to those handling the animal.

5.2 Specimen collection procedures

5.2.1 Blood specimens

Main specimen containers and devices

- Blood collection tubes with or without anticoagulants
- Venipuncture needles (various size depending on animal type)
- Syringe
- Swabs, cotton Dacron, or gauze-tipped swabs
- Soap/mild detergent
- Alcohol spray
- Appropriate transport media
- Animal shaving hair clipper machine
- Scalpel blade
- Screw cap or sealable containers
- Double-packaging materials
- Icepacks
- Labels
- Necropsy equipment

5.2.1.1 Collection of Blood from Cattle

Figure 5: Collection of blood from cattle [7]

Jugular Venipuncture

Using the halter, the head is elevated slightly, drawn to the side opposite the jugular vein to be sampled, and tied to a stationary surface. The vein is occluded by digital pressure in the jugular groove low in the neck. 18-gauge needle inserted into the distended jugular vein at approximately 45°. When positioned in the vein, blood is collected. When the desired volume has been collected, the occluding pressure is removed.

[7] Animal Resources and Care Division (2017). SOP: Blood Collection in Cattle. Virginia Tech University. USA. Retrieved May 19, 2022, from: https://ouv.vt.edu/content/dam/ouv_vt_edu/sops/large-animal/sop-bovine-blood-collection.pdf

Coccygeal Venipuncture

Blood collection from the coccygeal (tail) vein is performed with the animal restrained in a crush. The tail is held in one hand such that the ventral surface is accessible. The ventral surface of the tail is cleaned with a swab to remove faecal material. A needle is then inserted perpendicular to the skin surface on the midline between (approximately) the third and fourth coccygeal vertebrae. When blood flows from the needle, the syringe is attached, and the sample is collected. After sample collection is complete the tail is released. The syringe may be attached to the needle prior to insertion with gentle aspiration used to determine if the needle is in the correct location. Care must be taken as an untrained personnel risk sustaining kicks from cattle being sampled.

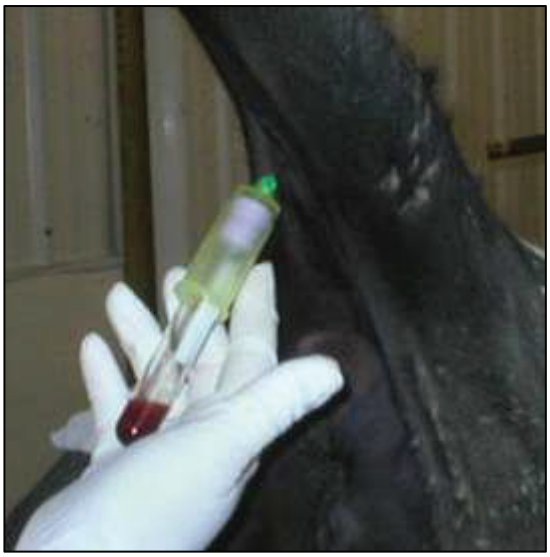

Figure 6: Collection of blood from cattle through coccygeal venipuncture[8]

5.2.1.2 Collection of Blood from Sheep

Correctly restrain the animal. Shave the area approximately 10 cm wide by 20 cm long. The assistant should turn the head of the animal at a 30-degree angle to the side by holding the animal under its jaw to allow for easy access to the vein Locate the vein and gently clean the area with water, mild soap and cotton removing any organic matter. Rinse the area with clean water. The easiest way to locate the vein is to draw an imaginary line from the middle of the animal's eye down the side of its neck. The vein can be located by applying pressure with the thumb or fingers below the half waypoint of the shaved area. The pressure will cause the vein to pop up and be easy to see. A small amount of alcohol poured over the area where the vein is supposed to be located.

Once the vein has been located, the area needs to be properly cleaned to keep bacteria out of the needle insertion site. This is accomplished by removing any organic debris using soap and water as previously described and applying alcohol spray on the area. Never go back over a place that has already been wiped, because bacteria could be carried back into the clean area. Once the area has been cleaned and the vein has been located, the blood can be drawn. This can be done by using a needle (20-gauge) and 5 cc syringe. If a needle and syringe are used, be sure to check that the needle is firmly attached to the syringe and that both the syringe and the

[8] Shabbir MZ, Ahmad A, Zahid MN, Nazir J, Akbar H editors (2013). Sample collection guide, a practical approach. Nexus Academic Publishers. Lahore, Pakistan. Retrieved May 19, 2022, from: https://nexusacademicpublishers.com/uploads/books/20140116135637.pdf

needle are new and clean. Contamination from other animals could cause contamination of the sample or

infection of the animal. Remove the cap from the needle and insert needle into the vein at the lowest point possible on the exposed area of the neck. By doing this, the vein can still be used if there are unsuccessful attempts at drawing the blood. Gently pull back on the syringe to see if the needle is in the vein. If no blood pulls back into the syringe, the needle is either parallel to the vein, or it has gone completely through the vein and out the opposite side. Light movements of the syringe can be used to try to locate the vein and penetrate it. When blood is easily pulled back into the syringe, the needle is within the vein. Fill the syringe with the desired amount of blood. Once the sample has been obtained, remove the pressure from the vein, take the needle out, and press gently *on the site of needle insertion. Finally, place the needle through the stopper of the appropriate blood collection tube.*

Figure 7: Collection of blood from a small ruminant through jugular venipuncture [9]

5.2.1.3 Collection of Blood from Swine

Restrain animal with snare, securely contained against a wall or corner, alternatively swine can be placed in a sling, smaller pigs can be held or placed in v-trough. Locate jugular groove and align with point of the shoulder and point of the manubrium. With bevel up, insert needle perpendicular to the skin. If needle is in the vein, blood will flow freely into tube. If vein is missed, the needle can be carefully repositioned, until vessel is penetrated. The vein is fairly deep and may roll away from needle. Typically, no more than two to three attempts should be made at a time to minimize distress to the animal and potential damage to the vein.

[9] Shabbir MZ, Ahmad A, Zahid MN, Nazir J, Akbar H editors (2013). Sample collection guide, a practical approach. Nexus Academic Publishers. Lahore, Pakistan. Retrieved May 19, 2022, from: https://nexusacademicpublishers.com/uploads/books/20140116135637.pdf

Figure 8 Collection of blood from a pig through jugular venipuncture [10]

Once collection is complete, remove vacutainer tube, then, applying pressure over injection site, remove needle. In order to ensure adequate hemostasis, apply pressure for 30 to 60 seconds.

5.2.1.4 Collection of Blood from Chicken

Cutaneous Ulnar or Brachial Venipuncture

Venipuncture of the cutaneous ulnar or brachial veins (wing veins) is superficial and easily visualized. Therefore, bleeding from these veins is usually the simplest and best method for obtaining blood from turkeys, chickens, and most fowl under field conditions. This is especially when the bird is to be returned to the flock. Have an assistant expose the vein to view by plucking a few feathers from the ventral surface of the humeral region of the wing. The vein will be seen lying in the depression between the biceps brachialis and triceps humeralis muscles. It is more easily seen if the skin is first dampened with 70% alcohol or other colorless disinfectant. To facilitate venipuncture, have an assistant extend the wing dorsally while you grip the brachio-ulna joint firmly together in the area of the wing web with the left hand. Puncture the vein of the right wing and collect blood. The animal care [11]and use guide for research animals requires limiting blood collection to no more than 10% of the bird's blood volume. Blood volume as a percentage of body weight averages 7%. A convenient calculation is to draw 1% of the body weight (i.e. 1 mL from a 100g chick).

Figure 9 Collection of blood from chicken through wing venipuncture[12]

[10] Norecopa: Norwegian Veterinary Institute (2022). Blood sampling pigs: Blood sampling, and Immobilization for Blood sampling. Norway. Retrieved May 19, 2022, from: https://norecopa.no/films-and-slide-shows/blood-sampling-of-pigs

[11] Source: Source : Deka A et al (2017) , Comparative Biochemical Parameters studies on Pati and Chara-Chemballi Ducks (Anas Platyrhynchos Domesticus during the laying periods. *International journal of livestock research Volume 7(2)*

[12] Kelly, L., Alworth, L. Techniques for collecting blood from the domestic chicken. Lab Anim 42, 359–361 (2013). https://doi.org/10.1038/laban.394

Jugular Venipuncture

The vein on the side of the outstretched neck is the jugular vein. Place the bird on a table or have an assistant hold bird with wings firmly held together with the body, setting it on its side. Stretch out the neck with one hand and part the feathers along the neck. The right jugular vein is usually larger. Place the needle at a slight angle, bevel up, against the vein. Puncture the vein and slowly withdraw blood. Remove the needle and apply pressure to the vein for a few seconds. Fill the appropriate vial 1/3 to 1/2 of its full volume.

Figure 10: Collection of blood from chickens through jugular venipuncture[13]

5.2.1.5 Collection of Blood from Horse

The jugular vein in the neck region of a horse is the best place to collect a blood sample. First, clean the jugular furrow of the neck with a piece of cotton or gauze pad soaked in alcohol. This will sanitize the area and make the vein easier to see. Restrain the animal with the head slightly elevated. The jugular groove is identified. The vein is occluded with digital pressure at the base of the jugular groove. A needle is inserted through the skin either with a firm thrust into the jugular vein or by gently easing the needle of 21-gauge through the skin and into the jugular vein at a 35° angle towards the head. When the desired volume is received, digital pressure may be removed, and the needle withdrawn from the vein. On completion of procedure observe

[13] Kelly, L., Alworth, L. Techniques for collecting blood from the domestic chicken. Lab Anim 42, 359–361 (2013). https://doi.org/10.1038/laban.394

animal for signs of excessive distress and treated if necessary. After collection of desired samples, apply disinfectant on the area.

Figure 11: Collection of blood from a horse through jugular venipuncture[14]

5.2.1.6 Collection of Blood from Dog

The cephalic and lateral saphenous vein is the most prominent spot for dog blood sampling. In some dogs, it may be needed to draw blood from jugular vein. Some dogs need to be restrained for blood sampling that it does not become a fight. Depending on the dog and sampler's experience, shaving might be required. When drawing a sample from the jugular vein, shaving is usually not required unless the dog has excessive amounts of hair. Occlude the cephalic or saphenous vein by placing hand so that the dog cannot move its leg back and applying pressure so that the vein will fill with blood and become more visible. Selecting the needle size should be based on the size of the dog and the size of the vein. Typically, a 22-gauge needle works for most blood draws. In larger breed dogs, an 18-gauge needle will be better, and the blood sample will be obtained much more quickly. Spray alcohol on the area that is about to be punctured by the needle as this will sterilize the area and prevent any bacteria from entering with the needle. Insert the needle of the syringe directly over the raised vein. If you have entered the vein correctly, a small amount of blood will enter the tip of the syringe. At this point, you should pull back on the syringe plunger and blood should begin to enter. Take 2-5 ml of blood at one time. Remove the needle and release pressure over the vein before doing this. Otherwise, blood will start coming out of where you just inserted the needle. Apply pressure over the venipuncture site for 30 seconds and then apply a disinfectant.

[14] Animal Resources and Care Division (2017). SOP: Blood Collection in the Horse. Virginia Tech University. USA. Retrieved May 19, 2022, from: https://ouv.vt.edu/content/dam/ouv_vt_edu/sops/large-animal/sop-equine-blood-collection.pdf

Figure 12 Collection of blood from a dog through lateral saphenous vein venipuncture[15]

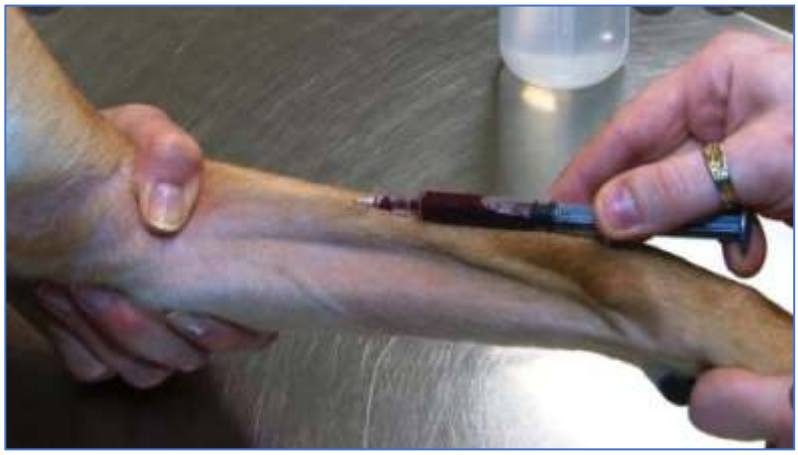

Figure 13 Collection of blood from a dog through cephalic vein venipuncture [16]

5.2.1.7 ***Collection of Blood from Cat***

The medial saphenous vein of the cat has a long straight course and is very superficial but other veins such cephalic or jugular also could be used depending on certain conditions. The saphenous vein is a good vein from which to collect small volumes of blood. The cat should be restrained in lateral recumbency by an assistant. Covering the eyes of the cat while restrained reduces agitation. Pressure is applied in the inguinal region to occlude

[15] Brashear, M (2011). Canine Blood Draw Lateral Saphenous. atDove Veterinary education. Retrieved May 19, 2022, from: https://www.atdove.org/video/blood-draw-lateral-saphenous

[16] Abrams, J (2012). Canine Cephalic Vein Blood Collection. atDove Veterinary education. Retrieved May 19, 2022, from: https://www.atdove.org/video/canine-cephalic-vein-blood-collection

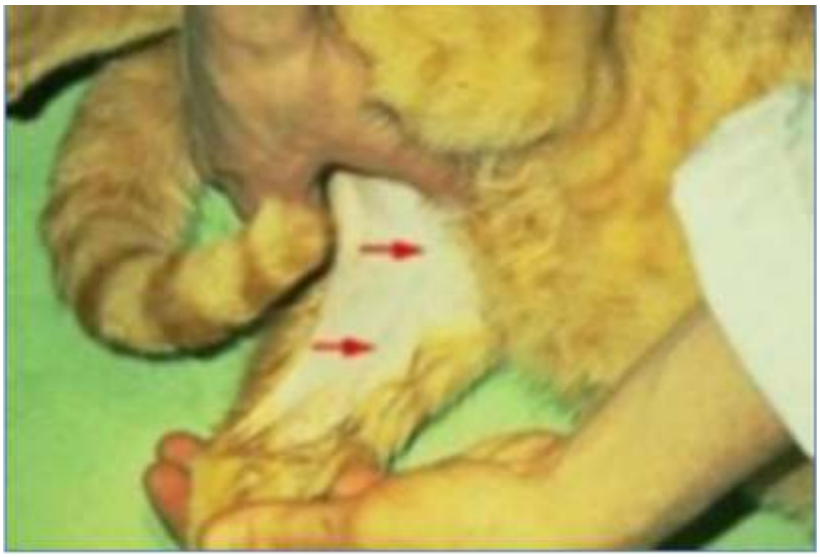

Figure 14 Location of the medial saphenous vein in cats and collection of blood through venipuncture[17]

venous return and cause the vein to engorge with blood. The site is wiped with alcohol and hairs are shaved. Use, 22- to 25-gauge needle attached to a 1 or 3 ml syringe for the collection of blood. Because the vein has a small diameter, vigorous aspiration will result in collapse of the vein. Therefore, only slight suction should be applied to the syringe when aspirating blood. The blood will flow slowly into the syringe; collect only a small volume (up to ~1ml) of blood. At the completion of the venipuncture, the needle is removed from the vein; the holder should release pressure from the inguinal region and place firm digital pressure at the puncture site for several minutes to prevent hematoma formation. Apply some disinfectant on the area after withdrawing blood.

5.2.2 Collection of Skin Scrapping

Skin problems and itchiness are common and frustrating disorders in animals. With so many underlying causes, finding the reason for the problem is important in order to find an appropriate treatment or even a cure. A skin scraping is a commonly performed sample that can help diagnose certain skin inflammations, fungal infections, and skin cancer and is quite effective in determining the presence of mites. A skin scraping is a collection of a sample of skin cells that are evaluated under a microscope. A skin scraping can reveal the presence of abnormal cells in the superficial layers of the skin. It can reveal certain fungi, bacteria, cancer cells and parasites. By determining the underlying cause of the skin disorder, an effective and appropriate treatment can begin.

[17] Shabbir MZ, Ahmad A, Zahid MN, Nazir J, Akbar H editors (2013). Sample collection guide, a practical approach. Nexus Academic Publishers. Lahore, Pakistan. Retrieved May 19, 2022, from: https://nexusacademicpublishers.com/uploads/books/20140116135637.pdf

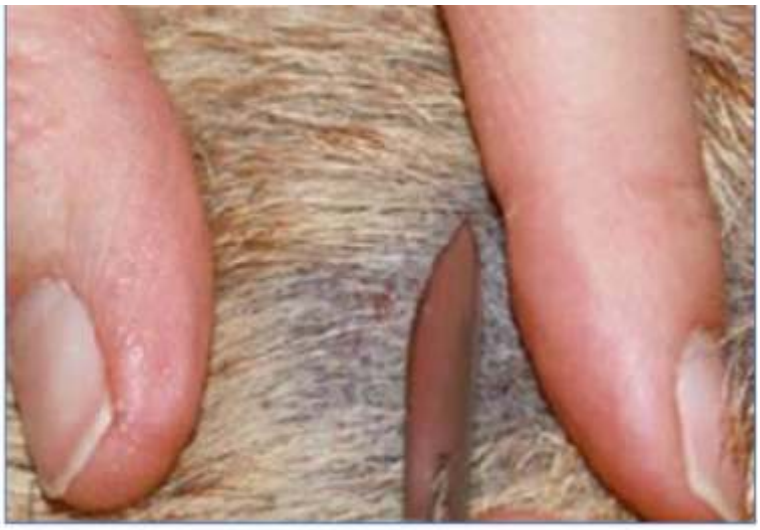

Figure 15: Collection of skin through skin scrapping [18]

A skin scraping is performed by collecting a sample of skin cells with the use of a scalpel blade. The blade is used to gently scrape layers of the skin, usually until a small amount of blood is seen, so that deep cells in the skin can be collected. This is important, especially if parasites are suspected since they often live deep in the skin. The skin cell sample is placed on a microscope slide, mixed with oil and evaluated under a microscope.

5.2.3 Collection of Fecal Sample

Faeces can be collected freshly voided or preferably directly from the rectum/cloaca for tests such as culture for microorganisms, parasite examination, or faecal occult blood determination; or can be collected for culture and molecular-based diagnostics from the rectum/cloaca using cotton, dacron, or gauze-tipped swabs, dependent on the volume of sample required by the specific test methodology. Samples collected on swabs should be kept moist by placing them in the transport media recommended for use with the specific test to be performed, which may range from sterile saline to culture media containing antimicrobials or stabilizers. Faecal specimens should be kept chilled (e.g. refrigerated at 4°C or on ice) and tested as soon after collection as possible to minimize the negative impacts on test results caused by death of the targeted microorganism, bacterial overgrowth or hatching of parasite eggs. Double-packaging of faecal samples in screw cap or sealable containers that are subsequently contained within sealed plastic bags will help prevent cross-contamination of samples and associated packaging materials. Faeces contained only in rectal exam gloves, plastic bags, or rubber-stoppered tubes are unsuitable as they are very frequently comprised by bacterial growth with gas production that can rupture plastic bags, displace stoppers, and allow leakage of the specimen.

Preferably, fecal samples should be collected from the rectum. If material is collected from the ground, it should be from the top of a freshly passed deposit. An appropriate sample size about 5g. Preferably submit a “golf ball” size sample in a zip lock type plastic bag or container that is airtight, watertight, and suitably robust. Samples should be submitted to the lab within 24 hours if possible. Store the specimens in a refrigerator until shipping and send with ice packs.

[18] Zoetis Inc (2014). Deep skin scraping. USA. Retrieved May 20, 2022, from: https://www.zoetis.ca/conditions/dogs/dermatology/deep-skin-scraping.aspx

Figure: collection of fecal samples [19]

5.2.4 Swabs

5.2.4.1 Genital tract

Ruminants: vaginal and urethral swabs

Carefully insert the soft tip end of the cotton- or dacron-tipped swab into the vagina about 2 inches (5 cm) past the opening of the vagina. Gently rotate the swab for 10 to 30 seconds, making sure the swab touches the walls of the vagina so that moisture is absorbed by the swab.

5.2.4.2 Respiratory

Nasal swab from small ruminants: Insert the cotton- or dacron-tipped swab gently and deep into each nostril, from 4 to 5 inches deep, gently rotate once or twice. Insert the same swab into the two nostrils. Discard the swab and use another one if it is dropped on the ground or touches another surface. Place the swab into the culture tube ensuring the top is secure.

Figure 17 Collection procedure of a nasal swab from small ruminants[20]

3.2.4.1.1 **Vaginal swab from small ruminants**. Spread the vulva lips and gently insert a dray cotton or dacron swab into the vagina. The swab wad should be inserted at least halfway into the vagina and rotated 180 degrees four or five times. Gently remove the swab and insert it into the culture tube.

[19] Royal Veterinary College (RVC). RVC/FAO Guide to Veterinary Diagnostic Parasitology. Fecal samples: Procedure. Retrieved May 20, 2022, from: https://www.rvc.ac.uk/review/parasitology/Faeces/Step1a.htm

[20] Alberta, Canada government (2020). Pneumonia in Bighorn sheep, collecting nasal swab samples. Retrieved May 20, 2022, from: https://open.alberta.ca/dataset/934c77f3-68ca-49f2-9e30-c4c584277d25/resource/c792620c-34de-426e-b39a-7f0a63796498/download/aep-bighorn-sheep-collecting-nasal-samples-2020-09.pdf

Figure 18 Collection procedure of a vaginal swab from small ruminants[21]

Poultry: To obtain the sample with minimal distress to the live bird, bird should hold against the chest with the wings folded (see photographs). An apron that cannot easily be ripped by the bird's claws should be used. Do not use the wings or neck to restrain the bird nor hold the legs and do not hold the bird upside down while carrying it.

Nasopharyngeal/choanal swab from poultry: Open the beak with the free hand, insert the swab into the choanal cleft (present in the upper beak) and gently rotate the swab to the mucosal wall for 10 to 30 seconds. Insert the swab into the culture or transport medium being careful not to touch any other surface.

Figure 19 Collection of oropharyngeal/choanal swabs from poultry[22]

Tracheal swab from poultry: Insert the swab gently through the glottis into the trachea and rotate the swab to the mucosal wall for 10 to 30 seconds. Insert the swab into the culture or transport medium being careful not to touch any other surface.

[21] Salinas J, Ortega N, Caro MR for HIPRA Laboratories for Animal Health. Abortions in shee: The role of Chlamydia abortus, 3: Diagnosis. Retrieved May 20, 2022, from: https://www.hipra.com/portal/en/hipra/knowledge/bgdetail/infectious-abortions-sheep/infectious-abortions-sheep-diagnosis

[22] Hy-Line International (2017). Proper collection and handling of diagnostic samples, Part 3: swabs. Retrieved May 20, 2022, from: https://www.hyline.com/Upload/Resources/TU%20SER3%20ENG.pdf

Figure 20 Collection of tracheal swabs from poultry[23]

5.2.4.3 Cloacal

Poultry. Expose the cloaca and gently introduce the cotton- or dacron-tipped swab into it through the vent. A gentle twirling motion often helps introduce the swab into the cloaca. Rotate the swab to the mucosal wall for 10 to 30 seconds. Insert the swab into the culture or transport medium being careful not to touch any other surface.

When submitting swab samples in liquid media such as brain-heart infusion broth (BHI), it is often recommended that the actual swab is not included in the tube. The proper procedure in this case is to introduce the swab in the media to wash off any material, and then roll the swab against the tube as it is pulled out to drain any excess fluid from the swab that may contain pathogenic material.

Figure 21 Collection of cloacal swabs from poultry[24]

5.2.5 Necropsy

A carcass with little or no decomposition is obviously one of the best diagnostic specimens. Carcasses intended for necropsy should be kept refrigerated, but not frozen, as freeze/thaw artifacts obscure gross and microscopic lesions. Necropsies should be conducted only by qualified veterinarians and pathologists. Para veterinary staff may be trained by veterinarians to conduct post-mortem examinations for specific purposes. The person conducting the post-mortem examination should have sufficient knowledge of anatomy and pathology to select the

[23] Hy-Line International (2017). Proper collection and handling of diagnostic samples, Part 3: swabs. Retrieved May 20, 2022, from: https://www.hyline.com/Upload/Resources/TU%20SER3%20ENG.pdf

[24] Hy-Line International (2017). Proper collection and handling of diagnostic samples, Part 3: swabs. Retrieved May 20, 2022, from: https://www.hyline.com/Upload/Resources/TU%20SER3%20ENG.pdf

most promising organs and lesions for sampling. The operator should wear protective clothing: overalls, washable apron, rubber gloves and rubber boots. Additionally, if potential zoonotic diseases are being investigated (e.g. brucellosis, mycobacteriosis, avian influenza and other), the post-mortem examination should be conducted in a biological safety cabinet. If this is not possible, an efficient face mask and eye protection should be worn. Arrangements should be made for appropriate safe disposal of animals and tissues. Samples of tissue from a variety of organs can be taken at post-mortem. Whether the necropsy is performed in a designated laboratory facility or in the field, appropriate biosafety and containment procedures should be followed to ensure operator safety and to provide non-contaminated and useful tissues for testing as well as to protect the environment and other animals from potential exposure to pathogens.

Importantly, the purpose of the necropsy is not only to collect specimens but to make informed observations regarding the pathology of the condition. It is useful to retain specialist veterinary pathologists to lead post-mortem investigations in important cases. Detailed procedures for conducting post-mortem examinations and tissue collection should be developed in laboratory and be available.

Dependent on the suspected disease, condition of the carcass and facilities available for necropsies, post-mortem specimens can be collected from one or multiple organs and submitted to the laboratory as either fresh (no preservative) or preserved specimens for further laboratory testing. The process of carcass autolysis can destroy diagnostically relevant tissues and infectious agents and so should be considered prior to collecting and submitting post-mortem specimens.

For fresh specimen particular attention must be paid to their handling and storage to avoid autolysis and overgrowth by bacterial and fungal contaminants. Ideally, freshly collected specimens are kept at a constant cool temperature from collection until processing for testing. Where such a cold chain cannot be provided fresh specimens for some test procedures can be collected into fluids such as ethylene glycol that inhibit the growth of secondary organisms. Preservation of post-mortem specimens is most frequently achieved by collection into formalin solution.

5.2.5.1 Necropsy sample collection for microbiological testing

Special transport media may be required for transport of samples from the field. Each piece of tissue should be placed in a fully labelled separate plastic bag or sterile screw-capped jar. Samples should be collected aseptically, and care should be taken to avoid cross-contamination between samples and ideally before any antimicrobial treatment is initiated. Sterile instruments should be used for collecting specimens for microbiological culture and care should be taken not to contaminate tissues with intestinal contents. Disinfectants should not be used on or near tissues to be sampled for bacterial culture or virus isolation. After collection, the samples for bacteriological examination should be refrigerated until shipped within 24 hours and should not be frozen. If shipment for virological testing cannot be made within 24 hours, samples should be frozen; however, prolonged storage at –20°C may be detrimental to virus isolation.

5.2.5.2 Tissue/organ samples collection for histopathology

To have a correct tissue evaluation, unfixed tissue should be handled gently, trying to avoid pinching or grasping the tissue too tightly. Try handling tissue by grasping areas adjacent to the tissue you desire to collect (e.g., manipulate intestine by holding onto the mesenteric fat instead of the intestine itself). If tissue is particularly bloody, rinse it with cold normal saline or PBS before fixation. To prevent autolysis and putrefaction, tissues must be fixed as soon as possible after cessation of blood flow. This is especially important for intestinal and central nervous

system tissues. Inadequate tissue fixation causes autolytic changes that may prevent proper tissue evaluation.

Fixation can be done by perfusion or by immersion fixation. Perfusion fixation followed by completion of fixation by immersion is the best technique but is not always feasible. For immersion fixation, neutral buffered 4-10% formalin in at least ten times (1:10) the volume of the tissue sampled should be used. The fixative needs to penetrate the tissue from all sides. Therefore, if tissue floats in the fixative, cover it with a piece of paper towel or gauze to ensure it is fully submerged at all times; if a large portion of the tissue rests on the bottom of the container, place some loosely arranged gauze under it.

Time of fixation depends on a variety of factors including the fixative used, the size of the specimen and temperature. For 10% neutral buffered formalin, the most commonly used and forgiving fixative, the time required to penetrate the tissue is approximately the square of the depth of the tissue (at room temperature). Thus, for a piece of tissue 5mm thick, the time to complete penetration is $5^2 = 25$ hours.

Some tissue types (e.g., skin, gut) curl when fixed, especially if fixed in a thin tube. Tissue cannot be straightened after fixation without damaging it so if it is important that the tissue remains flat for orientation purposes, small fragments of tissue can be placed on a piece of paper and then immersed in the fixative.

Store and pack formalin-fixed tissues separately from fresh tissues, blood and smears. Care should be taken to ensure that formalin-fixed tissues have not been frozen. Once fixed, tissues can be removed from formalin and, as long as they are kept moist and protected (e.g. by wrapping in formalin-soaked paper towels, then sealed in screw-capped jars), they can be forwarded to the laboratory without formalin.

5.2.6 Milk Samples

Milk can be collected from individual animals or from bulk milk in tanks pooled from multiple animals in a herd. The teat(s) used for sample collection should be cleaned, and any detergent thoroughly rinsed off before collection of the specimen. In collecting milk from individual teats, the initial stream must be discarded and only the subsequent streams sampled. The method of preservation prior to testing varies with the requirements of the test; in some cases it will be critical to avoid freezing or addition of chemical preservatives. For example: milk for serological tests should not have been frozen, heated or subjected to violent shaking, if there is going to be a delay in submitting them to the laboratory. Common sources of contamination are dirty teat ends, milk touching hands or fingers before entering tube, non-sterile tubes or inoculating needles, and excess alcohol on teat ends or hands. Milk samples should be collected and shipped in sterile vials or tubes.

5.2.7 Environmental and Feed Samples

Samples may be taken to monitor hygiene or as part of a disease enquiry. Environmental samples are commonly taken from litter or bedding and voided feces or urine. Swabs may be taken from the surface of ventilation ducts, feed troughs and drains. This kind of sampling is particularly important in hatcheries, artificial insemination centers and slaughterhouses in which specialized equipment is maintained. Samples may also be taken from animal feed, in troughs or bulk containers. Water may be sampled in troughs, drinkers, header tanks or from the natural or

artificial supply. In cases when a toxin or mineral deficiency is suspected in the environment or in the feed, samples can be collected for laboratory analysis.
Feed: A minimum of 1 liter /100g of feed should be collected, making sure it is representative of what the animal has been consuming.
Dried hay and bales: Sample a minimum of 8 bales or piles, collecting a minimum of ten (200g each) samples. Combined and mixed samples should be placed (300-350g subsample) into an airtight plastic bag.
Dried grains: "Hand-grab" samples (100g each) from ten separate locations within the bags, piles or bins should be collected. Combined and mixed samples should be placed (300-350g subsample) into an airtight plastic bag.
Fresh forages (plants): Fresh plants (stems and leaves only) should be collected into a paper bag to prevent it from growing mold. Contamination by soil or animal manure should be avoided.

5.2.8 Animal Sample Labelling

All samples should be clearly labelled with waterproof marker on appropriate primary containers. Regardless of the type of submission, a detailed sample information should be included with the samples to assist laboratory personnel in determining a diagnosis. A detailed sample information should include but not limited to:

- Owner's name/client number
- Sampling date/time
- Type of sample
- Sample number
- Species
- Breed
- Sex of animal
- Age of animal
- Animal identification
- Clinical signs (if relevant)
- Gross appearance (including size and location) of the lesion(s) (if applicable)
- Treatment (if any)
- Type of analysis requested
- Name of prescriber/sampler

If a zoonotic disease is suspected, this should also be clearly indicated on the submission form to alert laboratory personnel. The submission form should be placed in a waterproof bag to protect it from any fluids that might be present in the packaged materials.

5.2.9 Packaging and Storage of Samples for Shipment

The shipment of biologic specimens should comply with protocols established by the courier or shipping service used. In some instances, air transport requires compliance with IATA (International Aviation and Transportation Association https://www.iata.org/) regulations for hazardous materials. For example, shipments with fresh tissue samples should be clearly labeled as Biological Substance Category B. If high risk, reportable diseases are suspected, it is essential to follow the shipment precautions. Further details can be found at the International Air Transport Association website.
A fundamental approach is to devise a 3-layer barrier to protect the sample. The sample is placed in an appropriate primary container (sealed jar/bag/tube). This is then enclosed in a secondary container, which includes some adsorbent material. Note that items such as syringes,

obstetrical gloves, and containers without sealable openings are not suitable for shipment. Liquid samples should not ship in plastic bags; a sealable jar should be used. Waterproof markers should be used when labeling specimen bags and containers: the contents and client identification are critical information.

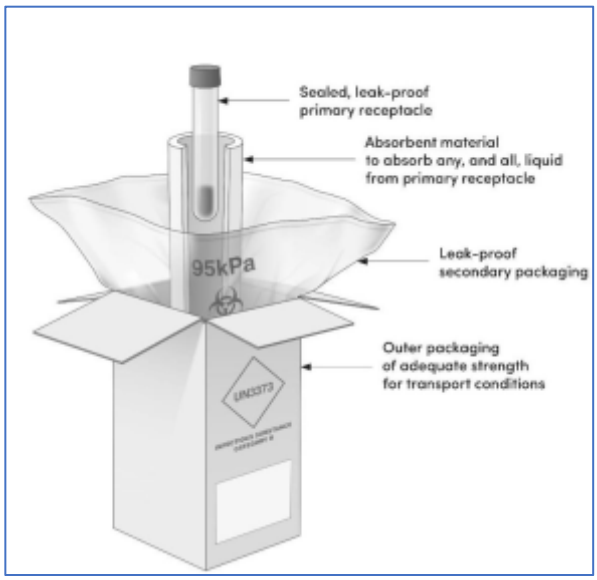

Figure 22 Packaging of biologic specimens for shipment using the 3-layer approach [25]

The secondary container is then placed in the shipping box (tertiary container), which often houses coolant packages as well as various cushioning materials (e.g., polystyrene foam) to protect the sample. The coolant materials should be sealed in plastic bags to prevent condensation damage. Coolant packs should not be placed directly onto samples, such as tubes of whole blood, that could suffer adverse effects if frozen in transit. Be sure to include the suitably protected submission form. The tertiary container is ideally a sturdy polystyrene refrigerator box or a cardboard box lined with a fitted polystyrene lining. If dry ice is used, this should be noted on the cardboard box label, and the lid should not be sealed with tape. Otherwise, CO_2 released from the dry ice could increase pressure and damage the package or contents.

Table 2 General overview of the type of analysis, the appropriate type of sample preservation and shipment condition

Type of analysis	Type of sample	Sample preservation	Shipment conditions
Microbiological culture	Organs, swabs, fluids (e.g. urine, aspirates, exudates), feces, carcasses	Bacterial transport medium (e.g. Amies' or Stuart's) into a sterile container	Refrigeration (~4°C)
Parasitology	Feces, organs, carcasses	Sterile container	Refrigeration (~4°C)
Virology	Organs, swabs, feces, carcasses	Virus transport medium	Refrigeration (~4°C) Possible freezing
Serology	Serum	Sterile and sealed vials	Refrigeration (~4°C). Possible freezing
Histopathology/	Organs (blocks of tissue	Neutral buffered 4–	Refrigeration (~4°C)

[25] *Triple packaging for UN 3373 (Source: Guidance on regulations for the transport of infectious substances 2019–2020. WHO/WHE/CPI/2019.20 World Health Organization 2019)*

Immunohistochemistry *	of maximum 0.5 cm thick and 1–2 cm long), carcasses	10% formalin in at least ten times (1:10) the volume of the tissue sampled	
Molecular analysis	Organs, swabs	Dry swabs, PBS	Refrigeration (~4°C). Possible freezing

* For certain suspected diseases (e.g. rabies), larger portions of brain are required; the brain is sectioned using a sagittal cut, half is submitted fresh, on ice, and the other half is submitted in 10% buffered formalin

6 Food, water and environmental samples

6.1 General food, water and environmental specimens' collection guidelines

This section is describing general procedures for sampling of food, water and environment. In order to be able to carry out the appropriate examinations on a sample and provide a meaningful interpretation of test results, it is essential that samples are collected using the correct procedure. Please contact the laboratory to obtain information and recommendations needed for specific details on sampling requirements (type, preservatives, handling procedures etc.). Also, laboratory staff can provide guidance when there are additional questions as well as offering assistance in interpretation of test results.

6.1.1 Request form and completion

A sampling request form should be completed for each sample. The following should be captured in a legible manner on the request form.

- Name and signature of organization/individual requesting for sampling
- Sample type
- Requested date of sampling
- Requested sampling site and community
- Tests requested

6.1.2 Sample identification

Each sample should be given a unique identity. When sampling many communities in a study or routine assessments, sample IDs can start with an abbreviation of the sampling community. . For example, samples from Tema could have codes starting with "TM". Sample containers should be marked with the Unique sample ID. Each sample should be accompanied by a sample identification sheet with the following information:

- Unique sample ID
- Sampling community
- GPS coordinates of sampling sites
- Name and signature of individual who performed sampling
- Sample type
- Date and time of sampling
- Destination of sample

6.1.3 Sample collection room or area

Sample collection sites should be free on any interference that can have an effect on samples collected. It should be the natural / 'every day' setting. There should be no modifications to the environment prior to sampling.

6.1.4 Collecting the samples

Specimens should be collected according to the instructions set out in the appropriate sections in this document or in specific laboratory SOPs. It is important to use appropriate and sterile primary sample collection containers. Adhere to recommended sampling times and climate conditions especially for environmental samples. For environmental samples it might be vital to perform sampling within different seasons to obtain a more wholistic outcome.

6.1.5 Labeling of primary sample' containers

All primary sample containers should be labeled with waterproof permanent markers. Barcodes may also be applied.

6.1.6 Sample packaging and storage

- **Primary receptacle**

This is a primary watertight, leak-proof and appropriate receptacle containing the samples. The receptacle is packaged with enough absorbent material to absorb all fluid in case of leakage and/or breakage.

- **Secondary packaging**

A secondary durable, watertight, leak-proof packaging to enclose and protect the primary receptacle(s). Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material shall be used to absorb all fluid in case of breakages. Secondary receptacles should not be overloaded with too many primary receptacles.

- **Outer packaging**

Secondary packaging is placed in outer shipping packaging with suitable cushioned material. Outer packaging shall be designed to protect their contents from outside influences, such as physical damages, while in transit. The outer packaging shall be appropriately labeled with the following:

a. Package markings (for e.g. biohazard symbol)
b. Proper shipping name
c. 'To' and 'From' labels

6.1.7 Sample transport and storage

Specimen should be transported at recommended temperature and in recommended containers. It is important to follow appropriate biosafety procedures including wearing of PPEs, using appropriate biohazard markings, using trained personnel etc.

6.1.8 Specimen acceptance and rejection criteria

Each laboratory shall define samples quality requirements and rejection criteria. The rejection criteria should be communicated to all laboratory users.
The following are examples of reasons for specimen's rejection:

- Incomplete request forms

- Request form not included with the specimen
- Specimen identification is missing or incorrect or does not correlate with the information on request form
- Unlabeled or improperly labeled specimen
- Specimens received in leaking, cracked or broken containers
- Specimens not appropriate for a particular test
- Specimens with obvious (visually, apparent) contamination
- Expired tubes or other collection device
- Incorrect temperature and/or packaging of specimen
- Stability of the analyte in the specimen has been exceeded (specimen is too old upon receipt)
- Inadequate volume or overfilling of specimen container
- Incorrect specimen container or tube
- Specimen not included with the request form

6.2 Specimen collection procedures

Main specimen containers and devices

sterile food-grade plastic seal bags/jars

This list is not intended to be exhaustive and not all items may be required for all types of sampling.

6.2.1 Food sampling

6.2.1.1 Containers for Samples for Analysis

Samples of food which are not pre-packed or opened cans or packets of foods should first be placed in clean, dry leak-proof containers such as wide-mouth glass or food quality plastic jars, stainless metal cans or disposable food quality plastic bags. Jars, bottles or cans should be suitably closed. Disposable food quality plastic bags should be sealed securely after filling so that they cannot leak or become contaminated during normal handling. Where necessary, it should then be placed in a second container, such as a plastic bag, which should be sealed save. Samples of alcoholic drinks should be placed in glass bottles.

Samples for microbiological examination should be taken and handled in a manner that eliminates the risk of contamination during the sampling process.

6.2.1.2 Quantity of Samples for Examination

The quantity of any sample procured should be such as to enable a satisfactory examination to be made. The quantity will vary according to circumstances but should normally be at least 100 grams. In any case of doubt the laboratory should be consulted.

6.2.1.3 Procedure for sampling foods

The sampling procedure may vary depending on the type of food and the reason for sampling, but generally, follow the next procedure:

- A sample, consisting of a specified number of sample units (usually five) drawn at random from each lot, shall be taken
- At least 100 grams or mL of food is usually required, unless an alternative quantity has previously been agreed with laboratory staff

- Collect original unopened container wherever possible. Where intact foods are to be examined, the whole sample in its original wrapping is placed inside a food-grade bag
- For aseptic sampling of open packs, take a portion of the food using appropriate sterile utensils. This will normally be a representative portion of all components but may be a specific portion such as a core sample, surface sample, filling etc. Place the food sample into a sterile food-grade bag or jar, taking care not to allow the sample to touch the outside or top edge of the container. Label the container with the location and sample details, unique reference/identification code, sampler name and date and time of sampling
- Record any relevant information such as the place of sampling, temperature of storage, type of packaging and type of sample on the laboratory sample submittal form
- Store samples in a cool box, preferably between 1 and 8°C (taking care to keep raw foods in a separate box from ready-to-eat foods, and hot food separate from cold), and return to the laboratory as soon as possible, preferably on the same day (unless there is a particular reason for a delay such as sampling late in the evening) but always within 24 hours of collection
- Do not allow sample units, that are usually frozen, to thaw during shipment

If necessary, samples can be left in a cool box overnight, provided that it is properly packed with an adequate number of cold packs or transferred to a secure fridge or cold-room, and submitted to the laboratory as early as possible on the following day.

6.2.2 Collection of water samples

Bacteria in water systems tend to be few in number due to low nutrient availability and are frequently associated with biofilms which form on the inside surfaces of pipework, valves etc. Higher counts will be found in water, which is stagnant or stationary for long periods, e.g. tanked supplies, infrequently used parts of buildings. It is therefore important to use a risk-based approach to the selection of appropriate sampling points, and to collect sufficient volumes of water to enable adequate assessment of the water quality. There are many available guidance documents give recommended volumes and methods for sampling. Disinfectants, such as chlorine dioxide, which are used to improve water quality, have residual effects and must be neutralized in order to give an accurate microbiological result. Therefore, appropriate sampling bottles containing neutralizing agents must be used and advice on these can be obtained from the testing laboratory prior to sampling.

It is important, in any investigation of a water system, to have a thorough knowledge of the supply and the system itself. In this respect, the local estates officers should normally be involved at an early stage. It is usually necessary to sample systematically, working proximally to the problem in order to identify its source.

Table 3 Sample bottles required for the collection of water for different microbiological and chemical analyses

Test Required	Sample Bottles
Coliform bacteria, Escherichia coli, Pseudomonas aeruginosa, Aerobic Colony Counts, environmental	1 x sterile 500 ml plastic bottle containing an appropriate neutralizer to neutralize any residual disinfectant in the water. The most commonly used neutralizer, which is appropriate for chlorinated or brominated water systems and those using ozone or hydrogen peroxide, is sodium thiosulphate. For mains water and hydrotherapy pools, 18 mg/L sodium thiosulphate should be added.

mycobacteria	However, for cooling towers, 180 mg/L (i.e. sufficient to neutralize 50 mg chlorine per litre) must be used. If alternative disinfection methods are used, the laboratory should be contacted to obtain the appropriate neutralizer, if one is available.
Legionella	1 x sterile 1-liter bottle Or 2 x sterile 500 ml plastic bottles Please note cold packs should not be used for Legionella samples. All water samples for Legionella examination should be stored at an ambient temperature (approximately 20°C), in the dark.
Endotoxin	Designated "Pyrogen-free" containers
Chemical parameters	Specific bottles should be requested from laboratory depending on tests required

6.2.2.1 Procedure for sampling tap water

The sampling strategy should determine the sampling technique. If the quality of water as delivered from the tap (i.e. including any bacteria that are colonizing the tap) is of interest, then the tap should not be sanitized and the sample should comprise the first portion of water delivered (i.e. omit steps i – iv below), preferably immediately after a period of no, or minimal, use. If only bacteria present in the system prior to the tap are sought, the tap should be sanitized and run for 2-3 minutes before sampling. When attempting to ascertain the origin of contamination, it may be appropriate to take samples before and after sanitization and flushing. The following sampling procedure should be followed:

I. If possible, ensure that the tap is in good condition, with no leaks
II. Remove any internal and external fittings such as hosing
III. Clean the end of the tap thoroughly with a clean disposable cloth (and detergent if necessary). Disinfect with sodium hypochlorite solution (sufficient to give 1% available chlorine) made up on the day of use, or chlorine dioxide foam. Sanitization can be carried out by preparing a hypochlorite solution in a measuring jug and suspending it under the tap, such that the end of the tap is immersed in the solution for 2 to 3 minutes. Alternatively, use a wash bottle to spray hypochlorite solution onto the outside and inside of the tap spout. Leave for 2-3 minutes before rinsing
Safety Note: Sodium hypochlorite is highly corrosive and should be handled with care. Nitrile gloves and goggles should be worn, and if contact with skin, eyes or clothes occurs, wash the affected area immediately with copious amounts of water. Contact with clothes may result in a bleaching effect. If a wash bottle is used, this should produce a directed spray but not a fine mist
IV. Turn on the tap gently to avoid unnecessary aerosol production and run water to waste for 2 to 3 minutes.
V. Label a sterile bottle (1 liter or 500 ml bottle containing neutralizer; see table above) with the location and sample details, unique identification, sampler, date and time of sampling
VI. Aseptically open the bottle, fill almost to the edge with water, replace and tighten the lid and shake the bottle to distribute the neutralizer
VII. Water samples (except for Legionella samples) should be stored between 1 and 8^{o}C. They should be submitted to the laboratory to ensure that they are examined promptly, ideally the same day, but always within 24 hours of collection

6.2.2.2 Procedure for sampling from water wells, tanks and swimming/spa pool water

Normally a single sample is taken. The most appropriate site for taking a single sample is where the water velocity is likely to be at its lowest and away from freshwater inlets or outlets. Depending on the size of the water well, tank or pool, it may be advisable to take samples from other sites to establish whether there are "dead spots" in the water circulation.

I. Wipe the outside of a sterile bottle (500 ml bottle containing neutralizer; see table above) with an alcohol wipe (this is not necessary if bottles are individually packed), and label with a waterproof marker (indicating the location and sample details, unique identification, sampler name, date and time of sampling)
II. Aseptically open the bottle
III. Immerse the bottle, keeping the long axis approximately horizontal but with the neck pointing slightly upwards to avoid loss of the neutralizing agent (see figure bellow)
IV. Once the bottle is immersed to about 200-400 mm (8-16") below the surface, tilt the bottle to allow it to fill, leaving a small headspace
V. On removal from the water, immediately replace the cap and shake the sample to disperse the neutralizing agent
VI. Water samples (except for Legionella samples) must be stored between 1 and 8°C, and submitted to the laboratory in a timely way to ensure that they are examined on the day of collection or at least within 24 hours of the collection
VII. If both routine testing parameters and Legionella are required, then separate 1 liter and 500 ml samples should be collected

It is good practice to determine total and combined disinfectant levels and pH value from the same site as the microbiological sample. These should be determined in a separate sample collected in a bottle without any neutralizing agent (e.g. a sterile plastic universal container) and the tests carried out at the poolside. These results together with information on the number of users in the pool at the time of sampling should accompany the sample to the laboratory. It is important to also note the type of disinfectant in use in the pool.

6.2.3 Environmental samples

6.2.3.1 Procedure for sampling freshwater (lakes, ponds, streams, rivers)

- Label bottle with a waterproof marker indicating the location and sample details, unique identification, sampler, date and time of sampling
- Samples near the surface can be taken by holding the collection bottle and lowering it into the water until covered. This handheld method of sampling, commonly called 'grab sampling', is the simplest way of collecting a water sample. The sample bottle should be held as shown in Figure 24 below techniques for taking hand-held grab samples
- On removal from the water, replace the cap
- Water samples must be stored between 1 and 8°C, and submitted to the laboratory in a timely way to ensure that they are examined on the day of collection or at least within 24 hours of the collection

6.2.3.2 Environmental surface sampling

Routine sampling of environmental surfaces is required in order to identify an environmental source of infection/contamination, to demonstrate efficacy of disinfection or cleaning procedures or as a research tool. It is essential that careful thought is given to the nature and purpose of the sampling and whether quantitative or qualitative results are needed.

The sites for collecting environmental samples are selected according to what information are trying to gather. For example, the goal may be to assess the effectiveness of sanitation procedures in an establishment; or goal may be to monitor the microbiological state of the environment while food is being prepared. The following are examples of sampling sites:

- food contact processing equipment, such as tables and conveyors
- non-food contact surfaces, walls, floors, and drains in processing areas
- raw ingredient handling areas
- finished product handling areas
- hospitals and laboratories
- fluid piping systems
- vacuum and air blower systems
- refrigeration units

Effective sampling of surfaces requires moisture for the microorganisms to adhere to the sampling matrix there, maybe moisture already present on the surface, or, more frequently, a sterile diluent such as saline or buffered peptone water is used. Appropriate neutralizers must be used if disinfectant residues are likely to be present on the surface to be sampled. Diluents and isolation media should be appropriate for the isolation of specific organisms. In some cases, it may be necessary to consider the need for controls or sampling over time to establish a baseline.

Sampling may be quantitative i.e., a known area is swabbed (using a swab or sponge) in a standardized way in order to compare results from different sites, or the same site but taken at different times. Alternatively, qualitative sampling (to determine the presence or absence of a pathogen) is usually appropriate when investigating the source of an outbreak or a cross-contamination incident. In this case, the larger the area sampled, the better the chance of detecting the pathogen of interest.

For large areas, sponges are often found to be most convenient, while cotton-tipped swabs are often more convenient for complex surfaces or areas which are less accessible. However, it should be noted that sponges generally achieve a more efficient recovery of micro-organisms than cotton-tipped swabs. Follow the general procedure outlined below to collect samples from surfaces.

I. Open a swab/sponge pack and aseptically take hold of the swab/sponge, either by holding the handle or by using sterile gloves for sponges without a handle. If not pre-moistened, moisten the sponge by dipping it into an appropriate liquid medium.
II. Applying a firm pressure and using up and down movements (taking approximately 1 second per stroke), swab the surface area.
III. Hold the swab/sponge at right-angles to the first movement and repeat the process.
IV. Aseptically return the swab/sponge to its sterile container.
V. Seal the container and label clearly indicating the location and sample details, unique identification, sampler, date and time of sampling
VI. Wipe over the area that has been swabbed with an alcohol wipe

Store the swab/sponge between 1 and 8^{o}C and return to the laboratory as soon as possible to ensure that it is examined on the day of collection or at least within 24 hours of collection.

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