Institut Pasteur du Cambodge	**MEDICAL BIOLOGY LABORATORY**	MAN-2017-002
	SAMPLING MANUAL	VERSION 16 Date of issue 10/03/2025

WRITTEN BY: S. KEO **DATE:** 20/06/2013	**VERIFIED BY:** S. KEO **DATE:** 10/03/2025	**APPROVED BY:** B. Guillard **DATE:** 10/03/2025
MODIFIED BY: B. Guillard **DATE:** 04/01/2025		
ADDRESSED TO: LBM		

REVIEWED ON		**BY**
UPDATED ON	**BY**	**NATURE OF MODIFICATIONS**
10/03/2025	Bertrand GUILLARD	Update for new test

TABLE OF CONTENTS

1. INTRODUCTION

This sampling manual aims to provide our patients, clinicians, nurses and healthcare workers with all the necessary information to perform a proper sample collection intended to be analyzed in the Medical Biology Laboratory (**MBL**) of the Institut Pasteur du Cambodge (**IPC**).

This manual contains information regarding proper patient preparation, sample collection timing, sample container type selection, sample transportation, and relevant patient clinical data critical for testing, timely reporting of results, and proper diagnosis.

MBL offers medical laboratory services per request from individuals and healthcare providers, including hospitals, doctors, nurse practitioners, clinics, laboratories, non-governmental organizations, and other health facilities that provide such services. Services include but are not limited to, all the tests listed in the updated catalog of IPC (ENR-2022-045), whose applicable version is always updated on the website www.pasteur-kh.org) performed on samples to provide information for the diagnosis, prevention, or treatment of a disease or medical condition.
MBL is composed of four activity areas and offers a panel of 169 analyses:

- **Blood Biology**: Biochemistry, Hematology, Immuno-hematology, Immuno-serology;
- **Microbiology**: Bacteriology, Parasitology, Mycology;
- **Mycobacteriology**: screening and therapeutic advice for tuberculosis and screening for non-tuberculosis mycobacteria;
- **Molecular Biology Platform**: screening and quantifying HIV, HBV, and HCV viral load, genotyping and resistance detection, screening for Sexually Transmitted and Respiratory infections.

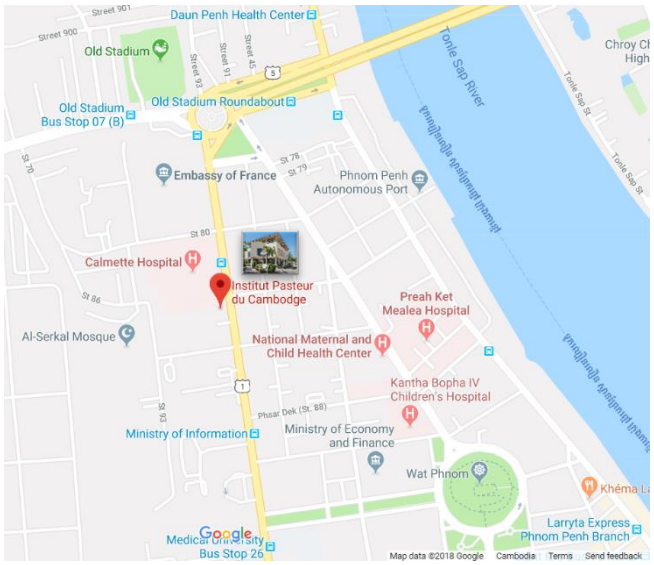
Quality Assurance system

- The MBL of IPC has set up a Quality Assurance system to meet your expectations and the required standard from the pre-analytical to the post-analytical phase.
- The reliability and accuracy of the test results are ensured by regular internal and external quality controls.
- The MBL is accredited by the French accreditation committee (COFRAC) according to the NF EN ISO 15189:2022 standard (accreditation n°8-4170, scope available on www.cofrac.fr)

Institut Pasteur du Cambodge

cofrac EXAMENS MEDICAUX Accreditation N°8-4170 Scope available on www.cofrac.fr

2. USEFUL INFORMATION



Institut Pasteur du Cambodge

5, Monivong Boulevard, BP 983, Phnom Penh

Mobile : +855 12 812 003

E-mail: accueil@pasteur-kh.org

Website: http://www.pasteur-kh.org

https://www.facebook.com/Institut.Pasteur.Cambodge

Public reception

Monday to Friday: ….7:00 am to 5:00 pm

Saturday: ……7:00 am to 11:30 am

"Special Opening Hours" during some public holidays, from 7:00 am to 11:30 am; notified by email.

Supervisory staff

Head of MBL	**Dr. Bertrand GUILLARD** bguillard@pasteur-kh.org
Mycobacteriology (Deputy Head of MBL)	**Dr. Sokleaph CHENG** csokleaph@pasteur-kh.org
Microbiology	**Dr. Puthea NOP** nputhea@pasteur-kh.org
Blood Biology	**Dr. Charya SITH** scharya@pasteur-kh.org
Platform of Molecular Biology	**Mrs. Seiha HENG** hseiha@pasteur-kh.org
Customer Service	**Mrs. Kheng Phally NONG** phally@pasteur-kh.org
Sampling Service	**Mrs. Sokuntheary KEO** ktheary@pasteur-kh.org

3. EQUIPMENT

MBL is fully equipped with modern equipment and automated devices.

➢ BLOOD BIOLOGY LABORATORY

- **Hematology**: 2 Pentra 80XL (HORIBA) for CBC, Automate SYSMEX CA-50 for hemostasis, 2 Minicap SEBIA Flex Piercing for hemoglobin electrophoresis, BD FACSCanto for CD4-CD8 lymphocytes count
- **Biochemistry**: 2 PENTRA C400 (HORIBA), Exias e1 for electrolytes and 2 Minicap SEBIA Flex Piercing for protein electrophoresis and HbA1c
- **Immuno-serology**: 2 COBAS Elecsys 411 (ROCHE) for serology, tumor markers, vitamins, and endocrinology

➢ MICROBIOLOGY LABORATORY

Bacteriology, Parasitology, and Mycology

- Microbial Identification: MALDI-TOF mass spectrometry (Biotyper, Bruker Daltonics),
- Automated zone size reader for antimicrobial disk susceptibility tests: ADAGIO Automated System (Bio-Rad),
- Automated microbial detection system for septicemia diagnosis: BacT/Alert (Biomérieux)

➢ MYCOBACTERIOLOGY LABORATORY

Liquid culture BACTEC 960 and BACTEC 320 (Becton Dickinson), GeneXpert IV (Cepheid)

➢ PLATFORM OF MOLECULAR BIOLOGY

Cobas 4800 (Roche), Light Cycler 480 II (Roche), T100^{TM} Thermal Cycler (Bio-Rad), GeneXpert GX-XVI (Cepheid), CFX Opus 96 (Bio-Rad), Kingfisher Flex System (Thermo Fisher) and TwinCubator (Bruker).

Our laboratory is equipped with a Laboratory Information System (LIS) which manages all stages in the handling of a biological file from sampling to sending results. Healthcare professionals can have access to the reports generated by the LIS via a results server.

4. SUBCONTRACTING

Analysis which are not carried out by the Medical Biology Laboratory of Institut Pasteur du Cambodge are mainly subcontracted to the Cerba Laboratory in France (accredited by COFRAC according to the NF EN ISO 15189, n°. 8-0945, Medical Examinations, https://www.lab-cerba.com).

LIST OF TRANSMITTED ANALYSIS (ENR-2024-001) is available on our website: http://www.pasteur-kh.org

5. MEDICAL PRESCRIPTION

Prescription sheet must be filled in accordance with the following guidelines:

These details are mandatory (under penalty of refusal of analysis).

They must be written legibly and not crossed out.

NB: oral prescriptions are not accepted.

1) Patient information

- First name and Last name
- Gender
- Date of birth
- Address, Phone number

2) Prescriber information

- Analysis requested
- Stamp of the prescriber
- Phone number and Email of the prescriber
- Signature of the prescriber
- Date of request

3) For samples delivered to Institut Pasteur

If the sample was taken outside IPC, the sampler must provide us with the following information:

- Date and time of the sample collection
- Identity of the person collecting the sample
- Useful clinical information for requested examinations

4) Modification of prescription

Modification of prescription by phone is not permitted.

A new written prescription must be sent to the laboratory.

These analyzes will be performed:

- On a tube kept in the laboratory if the prescription reaches the laboratory before 48 hours (sample retention period) and after verification of the nature and stability of the primary sample.
- On new sample if the prescription is made later.

5) Prescription sheet

PRESCRIPTION SHEET of MBL (FOR-R1-005) is available on our website: http://www.pasteur-kh.org

6. CLINICAL INFORMATION

Some analyses require additional information for the correct interpretation of the results:

	Clinical Information to obtain
Biochemistry	• Clinical diagnosis • Medical treatment
Hematology	• Known pathology
Immuno-hematology	• Current pregnancy. In case of prophylaxis by injection of Immunoglobulin anti-D (Rhophylac®), specify the date and the dosage of the injection. • Transfusion history and date of last transfusion
Hemostasis	• Blood test before hospitalization • Assessment for hemorrhagic syndrome (thrombosis) • Anticoagulant therapy, dosage and target
Hormonology	• Date of last period • Suspected ectopic pregnancy • Sampling time: preferably in the morning unless ordered by the doctor
Tumoral markers	• Current treatment • Pathology
Drug monitoring	• Drug Name • Dosage • Date and time of last dose • Date and time of collection • Clinical context
Malaria search	• Presence of fever / Clinical signs • Travel • Prophylactic or curative treatment taken • Antecedents

For patients treated with high doses of biotin (> 5 mg/day), interference is possible for the laboratory immunoassays carried out in the laboratory. It is recommended that patients who have consumed high doses of biotin wait a minimum of 3 days before having blood collected for laboratory immunoassays (in particular thyroid-function tests).

7. INFORMATION SHEET

Some analysis requires specific information in addition to prescriptions:

Analysis	REQUIRED INFORMATION (The forms are available on our website: http://www.pasteur-kh.org)
Blood grouping	Sample for blood grouping (FOR-R1-009)
Malaria	Sampling for Malaria testing (FOR-R1-006)
Bacteriology	Clinical information for bacteriological examination (FOR-DST-005)
Blood culture	Blood culture form (FOR_R1-014)
Mycology	Mycological sampling (FOR-R1-008)
Leptospirosis	Information sheet for Leptospirosis diagnosis (FOR-R1-007)
Dengue Fever	Information sheet for Arboviruses suspected patient (FOR-R1-017)
Viral load (HIV, HBV, HCV)	Viral load test request form (FOR-R1-011)
HIV-1 drug resistance	HIV-1 ARV Drug resistance testing request form (FOR-R1-012)
HBV, HCV genotyping	HBV HCV Genotyping test request form (FOR-R1-013)
COVID-19 PCR	Lab Request Form (Ministry of Health)
ANATOMO-PATHOLOGY	Request for Anapathology exam (FOR-R1-020)

8. SAMPLING REQUIREMENTS

The fulfilment of the following requirements is mandatory to obtain a reliable analytical result and the guarantee of the patient-specimen link, which is essential for the quality of the results

General Specimen Collection Guidelines

- During specimen collection wear appropriate personal protective equipment
- Sampling must be performed with disposable equipment.
- The tubes must be filled optimally.
- Make sure screw-cap lids are fastened evenly and securely to avoid leakage.
- Use leak-proof containers and plastic zip-lock IPC transport bags that have a separate outside compartment for the test request form.
- The material used and the waste generated by sampling must be separated into potentially contaminated waste and other waste similar to household garbage.
- Sharps material should be collected in adapted containers (e.g. needle collector).

Sample identification

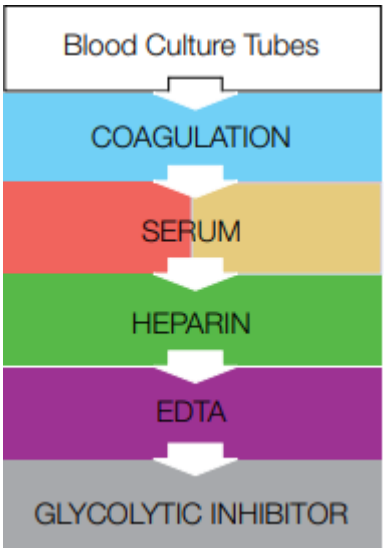
- Identify all the samples with the name, the sex, and the date of birth.
- A final check of the information labeled on the samples must be carried out by asking the patient to identify himself.
- Tubes for blood grouping must include: name, date of birth, date of collection (see below 7.1.2).
- If the patient is under treatment, mention it on the prescription sheet: identify the nature (anticoagulant, antibiotics, antiretrovirals, etc.), and the dosage.
- Do not forget to sequentially number the vials if there are several (provoked hyperglycemia, sputum, etc.).
- The identification label of the patient must be affixed so that the information is perfectly readable by the instruments' barcode readers.

Nature and order of the tubes

Due to the high sensitivity of modern testing, proper collection is essential to specimen integrity. Some tests require whole blood, plasma, or serum for testing. The collection tubes contain different types of additives, which are specific to the individual test(s).

The draw order for specimen tubes is as follows:

1) Blood culture
2) Blue tube for coagulation (Sodium Citrate)
3) Serum tube = Red without Gel or Yellow SST tube (Serum-separative tube)
4) Green and Dark Green tube (Heparin, with and without gel)
5) Purple tube (EDTA)
6) Gray tube (Oxalate/Fluoride)



If blood is drawn using a syringe, transfer the specimen into the appropriate collection tube(s) by puncturing the rubber stopper with the syringe needle and allowing the blood to be drawn into the tube by the vacuum.

These guidelines must be followed to maintain the specimen's integrity, providing optimal results.

Available consumables

Requests for the supply of consumables should be sent to the reception of the MBL.

Requests are processed within 48 hours.

The material can be obtained from the nurses or by the courier.

For all consumables requests, the applicant must complete and sign a form available at the reception of IPC**.**

It is important to respect the correct storage conditions: at room temperature, out of direct sunlight.

Any expired material must be returned to the courier of IPC.

9. THE SAMPLES

Instructions about samples collected by the patients themselves, such as **urines** (FIT-R1-002), **stool** (FIT-R1-003), **sputum** (FIT-R1-005), and **semen** (FIT-R1-004) are available on our website: http://www.pasteur-kh.org.

In case of non-conformity (prescription or samples) the prescriber or the patient will be informed.

A new prescription or a new sample will be requested.

9.1 Patient status and information

9.1.1 Food status

- Lipid profile (triglycerides, total cholesterol, HDL and LDL) must be necessarily taken from **a fasted patient for 12 hours**.
- Blood glucose must be necessary taken from a fasted patient for 8 hours.
- It is recommended (but not mandatory) to be fasting for immuno-enzymology (serology, hormonology, tumoral markers,…), hematology and hemostasis.
- The patient may be allowed to drink a glass of water and take their usual medication, unless drug dosage.
- It is recommended not to have smoked or chewed gum.

9.1.2 For the determination of Blood Group

- MBL in IPC applies an identity vigilance system for monitoring and preventing errors related to the identification of patients. In this context, only blood samples taken at IPC are accepted for the blood group.
- The patient's identity is systematically verified by the MBL secretary and then by the MBL nurses using an official document (ID card, passport, birth certificate, family record book or driving license). A copy of this document is scanned in our Laboratory Information System.
- Before sampling, the nurse must verify the identity of the patient by open questions, in Khmer or in English:
- “Spell your family name? Spell your first name? What is your date of birth? “
- Correct identification of the patient is the responsibility of the MBL nurses, having taken the blood.
- Blood group technique is performed according to two different techniques by two different technicians.

9.1.3 For the determination of prolactin

It is recommended that the patient is **at rest 20 minutes** before the blood test.

9.1.4 For the determination of cortisol

Unless it is specified on the prescription, the sample must be taken between **8 am and 10 am**.

9.1.5 Sperm (Spermogram, Spermoculture)

The sampling must be done in the laboratory, only in the morning between **7 am and 11:00** am (spermogram is not performed on Saturday).

9.1.6 Request from insurance or in a legal context

For a medical analysis report carried out in a legal or insurance context, the secretary and then the nurse must verify, using an official identity document with photo, that the patient presenting is the one concerned by the request of examination. A copy of this document is scanned in our Laboratory Information System.

9.2 Blood samples

9.2.1 Blood collection: Venipuncture

- Identify the tubes with label,
- The sampler must disinfect his hands by washing with soap or using a hydro-alcoholic solution,
- Wear gloves,
- Place the patient's arm in the low position,
- Place a clean tourniquet 7-10 cm above the elbow, do not leave it on more than 1 minutes,
- Identify the veins by palpation,
- Asepsis of the venipuncture site (do not touch it again),
- Remove the needle shield,
- Tighten the skin,
- Puncture the vein (the needle must form an angle of approximately -30° with the arm),
- As soon as the needle is in place, push the first vacutainer tube into the large end of the hub body penetrating the stoper: the blood should flow into the tube, the tourniquet can be loosened (in case of difficulty, have the patient clench his fist),
- Wait until the tube is completely filled (blood flow stops) before changing the tube,
- Respect the order of tube collection,
- Homogenize the tubes by slow inversions (minimum: 8 to 10),
- After removing the last tube, remove the needle and apply a dry cotton ball to the puncture site,
- Have the patient maintain firm pressure for at least 1 minute,
- Meanwhile, dispose of the dirty needle in an adapted container (needle collector),
- NEVER RECAP A NEEDLE +++,
- Disinfect the outside of the tubes if they are soiled.
- Apply a Band-Aid,

Never transfer the blood collection from one tube to another: anticoagulants/additives specific to each tube would be mixed, impacting the analyses carried out +++

9.2.2 Biochemistry

1°) Tubes

- For blood lactate and glycemia levels, use an oxalate tube K / Na fluoride (inhibitor glycolysis)
- For other biochemical analysis, use lithium heparin tube:

2°) Specific analyses conditions:

- For Electrolytes (Na, K, Cl): samples must arrive within 4 hours after collection, hemolyzed samples are unacceptable.
- For glucose: analyses must be performed less than 24 hours after collection on gray tube (fluoride).

9.2.3 Oral Glucose Tolerance Test (OGTT)

The OGTT can be used to screen for impaired glucose intolerance (prediabetes) and diabetes mellitus. The fasting sample should be taken and the time point should be notated. The patient should then consume the correct amount of glucose asked by the medical doctor.

On the day of the test the patient must arrive in a fasting state.

A fasting glycemia sample is taken to establish a baseline glucose level. Then, the patient will drink the glucose (50g, 75g or 100g).

Samples are then taken at various timepoints ending at either 60 or 120- or 180-minutes post-consumption of glucose.

Patients are asked to fast throughout the test except for drinking the glucose. Throughout the test, patients should remain inactive.

Drinking 50g of glucose:

- Fasting sample
- Additional sample taken at 60-minute

Drinking of 75g of glucose:

- Fasting sample
- Additional sample taken at 60-minute and 120-minute

Drinking of 100g of glucose:

- Fasting sample
- Additional sample taken at 60-minute, 120-minute and 180-minute

9.2.4 Fasting and After Meal blood sugar

1. First sample when the patient is fasting.
2. Second sample 2 hours after the start of the lunch or the breakfast rich in sugar.

9.2.5 Serology

- Use dry tubes: 

9.2.6 IGRA Test (Interferon gamma release assay test; QuantiFERON-TB Gold)

Only for patients aged > 5 years

If not collected at IPC:


- 2 lithium-heparin tubes must be taken for each test:
- Gently mix by inverting the tubes several times
- Label the tubes with the time and date of the blood collection
- There are 2 possible options for the tubes:

Option 1: The tubes are maintained at room temperature (22°C ± 5°C) for no more than 16 hours from sampling to arrival at IPC.

Option 2: The tubes are maintained at room temperature (22°C ± 5°C) up to 3 hours after blood collection; then they may be refrigerated (2-8°C) up to 48 hours before arrival at IPC.

9.2.7 Complete Blood Count (CBC), Blood smear, Reticulocytes

- Use tube EDTA:
- For Complete Blood Count (CBC) and Reticulocytes: samples must arrive within 24 hours after collection.
- The morphology of blood cells by microscopic examination requires that the blood smear is prepared within 6 hours of blood collection.

9.2.8 CD4/CD8 lymphocyte count

- Use tube EDTA:
- Preferably take the sample on the day of arrival in IPC (tolerated pre-analytical delay of 24 hours at room temperature).
- A blood count (CBC) will systematically be performed and billed in order to obtain a lymphocyte count on the same sample.

9.2.9 Glucose-6-phosphate dehydrogenase (G6PD)

- Use tube EDTA:
- Preferably take the sample on the day of arrival in IPC (tolerated pre-analytical delay of 24 hours).
- Not to be performed in the event of transfusion (<3 months) or hemolysis.
- A blood count (CBC) will systematically be performed and billed in order to calculate/interpret the requested analysis.

9.2.10 Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT)

1°) Tube

Use citrated tube: 

2°) Special sampling conditions:

- The tourniquet may not be tight and must be maintained less than 2 minutes (coagulation activation).
- Never collect after a heparinized tube.
- The puncture site should be away from any perfusion.
- The tube must be filled appropriately as indicated by fill mark on label
- Gently invert the tube 4-5 times immediately after blood collection.
- Conservation conditions:

In whole blood a sample can be kept until 24 hours (for Prothrombin time/PT/TP/INR) or 6 hours (for Activated Partial Thromboplastin Time/APTT/TCK) after collection (storage at room temperature).

9.2.11 Viral loads and Genotyping (HBV/ HCV/HIV)

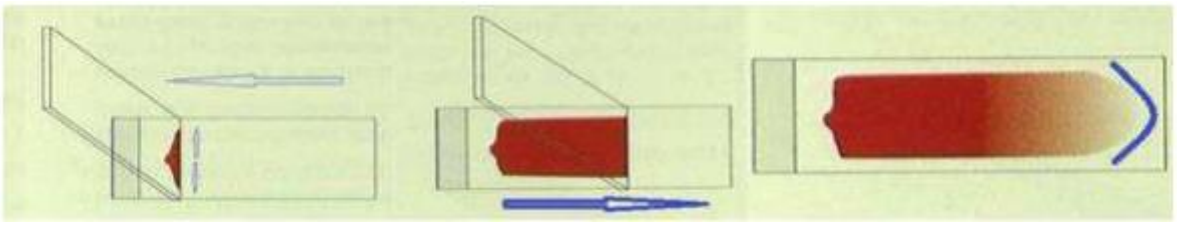
- 2 EDTA tubes whole blood must be taken for the viral loads and HIV genotyping.
- 1 EDTA tube whole blood must be taken for HBV genotyping and HCV genotyping.
- Conservation conditions: EDTA tubes whole blood (stored and/or transported at 2°C to 25°C) must arrive in IPC within 24 hours after collection.

9.3 Myelogram

Bone marrow smears are performed at the patient's bedside.

Spread the drops deposited on the slides using another slide tilted at 40° as for blood smears.

A good quality smear does not reach the end of the blade and leaves a few millimeters free along the side edges.



At least two slides are air-dried without ventilation or agitation, identified at the patient's bedside, before being sent to the laboratory wrapped and accompanied by the prescription form and the clinical context.

Clinical information will be required: investigation of cytopenias, investigation of abnormal peripheral blood smear morphology, investigation of organomegaly, investigation of bony lesions on radiological imaging…

For any request for Myelogram, a blood count (CBC) will systematically be performed and billed in order to interpret the requested analysis.

9.4 Urine samples

9.4.1 Urine sample for biochemistry

- Use a suitable small container (available in the laboratory).
- Properly close the urine container.
- Disinfect the outside of the container if they are soiled.

9.4.2 24-hour urine (not collected at IPC)

- Identify the bottle with last name, first name, date of birth, date of collection
- On the first day, when you wake up, empty the entire bladder into the toilet and note the hour of the beginning.
- During the next 24 hours (day and night), collect all your urine in the bottle (preferably to keep cool) including one last time the next morning at the same time, previously noted. Bring the bottle to the laboratory within 2 hours after the end of the collection.

9.5 Microbiological samples

Collect before any antibiotic therapy +++ (bacteria are detected more commonly if specimens are collected before administering antibiotic therapy, leading to improved pathogen detection and targeted antibiotic therapy).

Before taking samples, the sampler must disinfect his hands by washing with soap or using a hydro-alcoholic solution then put on gloves.

After taking the samples, identify carefully the specimens.

9.5.1 Urine sample for urine culture

Urine is preferably collected in the morning and/or after at least 2 hours of bladder stasis.

Mid-Stream Urine (not at the beginning and not at the end) should be collected.

Ensure proper care to clean the genitals before collection.

Follow these steps to get the sample:

- The first few drops of the urine should be discarded in the toilet.
- Mid-stream urine sample should be collected in the sterile container provided.
- Latter part or the end of the urine should not be collected.
- Close the container.
- Bring the specimen to Institut Pasteur no later than 2 hours after collection if sample kept at room temperature, no later than 12 hours if sample refrigerated.

Upon request, IPC can provide containers with Boric acid preservative that can be brought to Institut Pasteur within 48 hours after collection of urine (mid-stream urine after intimate hygiene).

For the research of mycobacteria (BK), mycoplasma or chlamydia, collect the first jet of urine in the morning. For the research of BK, the sampling is done on 3 consecutive days (in 3 separate pots).

9.5.2 Urine collection for the detection of Schistosoma eggs

Collect the entire first morning urination or a complete urination performed after physical effort (sustained walking, going up/down stairs, etc.) respecting a bladder stasis of at least 2 hours. The physical effort aims to unhook the eggs stuck in the bladder mucosa.

Identify the bottle with name and date of birth. Note the date and time of collection.

Bring the sample quickly to the laboratory.

9.5.3 Sputum

- Use sterile sputum bottles provided by the laboratory.
- Respect the instructions provided by the laboratory.
- A volume of 2 to 4 mL is recommended. A volume less than 0.5 mL is not exploitable except for direct smear microscopy or in the case of purulent sputum.
- Close the vials tightly.
- Disinfect the outside of the bottles if they are soiled.
- Bring the specimen to Institut Pasteur, at room temperature, no later than 2 hours after collection.

These samples cannot be made in the laboratory; they must be done at home, in the open air, away from other people - and not in confined spaces such as toilets.

<u>Three-morning sputum samples</u> collected within 3 days are recommended for diagnosis of Tuberculosis.

9.5.4 Stool

- If possible, this examination should be carried out during diarrheal episodes.
- Identify the bottle with name, date of birth, date, and time of sample.
- Collect the stool in the sterile vial provided by the laboratory.
- Close the bottles correctly.
- Disinfect the outside of the vials if they are soiled.
- Bring the sample to the laboratory within 2 hours of collection if sample kept at room temperature; within 12 hours if sample kept refrigerated.

For stool parasitology: Do not put the samples in the refrigerator, bring the sample to the laboratory

9.5.5 Blood culture

For bacteria screening: use BACTALERT vials

Conventionally, 2 to 3 pairs of blood cultures (1 pair: 1 Aerobic vial + 1 Anaerobic vial) should be taken 30 minutes apart at the time of the feverish peak, before any antibiotic therapy (+++). Nevertheless, according to the latest recommendations, it is now accepted that 2 to 3 pairs of blood cultures can be collected in a single sample, if the vials are correctly filled (10 ml of blood per vial).

In case of suspicion of infectious endocarditis, take 3 pairs of blood cultures obtained by 3 venous punctures spread over 24h and spaced at least 1 hour.

- Take by venipuncture after strict asepsis of the sampling site by using 70% alcohol plus iodine product. The venipuncture site is not fully clean until the disinfectant has fully evaporated.
- Inoculate vials for blood culture after disinfection of the cap with 70% alcohol or iodized product. Allow bottle tops to dry to fully disinfect.
- Take a sufficient quantity of blood (+++):
  - 10 mL per vial in adults,
  - In children, the recommended volume of blood to collect should be based on the weight of the patient (see Table 1), and an aerobic bottle should be used, unless an anaerobic infection is suspected.
- When a set of an aerobic and an anaerobic bottle is used:
  - If using needle and syringe, inoculate the anaerobic bottle first.
  - If using winged blood collection set, inoculate the aerobic bottle first.
- Immediate transportation to the laboratory at room temperature.

Table 1. Blood volumes suggested for cultures from infants and children

Weight of patient (kg)	Volume for culture per bottle (ml)
≤1	0.5 - 2
1.1-2	1.5 - 4.5
2.1-12.7	3 - 6
12.8-36.3	5[a]
> 36.3	10[b]

[a] Two pairs of blood culture are recommended.

[b] Three pairs of blood culture are recommended.

9.5.6 Vaginal sample

The patient must avoid any intimate toilet and sexual intercourse in the 24 hours preceding the exam. It is better to avoid sampling during the menstrual period because the flora is modified (unless otherwise advised by the prescriber). Prior to sampling, the sample collector must check the physiological conditions of the subject (pregnancy, virginity, etc.). The sample at the cervix level for the research of intracellular pathogens (Chlamydia, mycoplasma, HSV, etc.) is carried out with specific swabs by performing 3 or 4 rotations to collect as many cells as possible.

The vaginal sample must be taken before or after any antibiotic treatment:

• 15 days for Chlamydia,

- 5 days for common germs
- 3 days for treatment with vaginal ovules

- The sample consists of a collection of vaginal secretions. It is done on swabs and as much as possible at MBL.
- Ideally 3 swabs should be taken (2 swabs for the vaginal wall and 1 for the endocervix examination).
- If joint request for Chlamydia and Mycoplasma, 2 rayon or Dacron swabs for the endocervix.
- Clinical information to be provided: date of last menstruation, current treatment, recent or old history.
- The samples must be transported to the laboratory at room temperature within < 1 hour.

Upon request, IPC can provide swab with transport media for a better conservation of bacteria (Gono ++): the transportation to the laboratory is then possible within 24h at room temperature.

9.5.7 Urethral sample

No local treatment, no personal hygiene before sampling. The sample is carried out if possible in the morning before urinating, if it's not possible the patient should not urinate in the two hours preceding the sample.

The urethral sample must be taken before or after any antibiotic treatment:

- 15 days for Chlamydia,
- 5 days for common germs

It is better that the sample is taken in the laboratory.

- In case of urethral discharge, collect and spread the serous liquid on 2 slides.
- Systematically:

  1) Take a sample using a fine cotton swab and inoculate immediately on the agar plates (Chocolate agar and Chocolate + VCN agar). For the detection of Chlamydia and Mycoplasma, cells should be obtained by scraping with a dacron swab.

  2) Take a second sample for direct examination (if there is no discharge)
- Transport to the laboratory must be carried out at room temperature within < 1 hour.

Upon request, IPC can provide swab with transport media for a better conservation of bacteria (Gono ++): the transportation to the laboratory is then possible within 24h at room temperature.

9.5.8 Pap smear sample

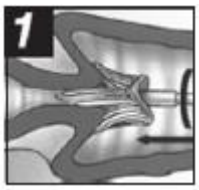
Must be done:

- Outside of menstruation or bleeding
- Away from sexual intercourse (48 hours)

Cannot be done:

- In case of vaginal medication, vaginal contraceptives, vaginal creams, vaginal jellies, or douches during the 48 hours before the exam.
- In case of vaginal infection (wait a month)

Obtain an adequate sampling from the cervix using a broom-like device. Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently, and rotate the broom in a clockwise direction five times.



Rinse the broom as quickly as possible into the solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device.

Tighten the cap.

Record the patient's name and ID number on the vial

9.5.9 Ear, Nose and Throat sample

- Throat sampling

The sample is preferably taken at least 2 hours after the last meal.

Swab:

- Tonsils or pillars (bilateral sampling)
- Inflammatory or necrotic areas.
- At the periphery of any false membranes (suspicion of diphtheria)

- Tongue / mouth sampling

  For the explicit search for *Candida spp*, take the sample from the tongue, palate and of the internal face of the cheeks by swabbing.

- Ear sampling

  External ear: The sample is taken with a swab at the level of the canal by pressing on the walls.

  Middle ear: After cleaning the external ear canal, the sample is taken by the ENT doctor after paracentesis by swabbing the fluid collection or after aspiration (collection of pus in a sterile vial).

- Nose sampling

  Swab the 2 nostrils (lower third), with the same swab which can be moistened with physiological water.

9.5.10 Puncture fluid (not collected at IPC)

Fluids (joint fluid, pleural fluid, ascites, etc.) should be taken prior to any local or general antibiotic or antifungal treatment (therapeutic window of at least 5 days).

After surgical disinfection, aspirate biological fluid using syringe, introducing as few bubbles as possible (think after to remove the needle and seal with a stopper).

Transport of the samples (put in syringe after removing the air / sterile container / no additive tube) to the laboratory must be carried out at room temperature within <2 hours.

9.5.11 Pus from abscess (not collected at IPC)

Pus is preferred to a swab.

Samples should be taken prior to any local or general antibiotic or antifungal treatment (therapeutic window of at least 5 days).

After surgical disinfection, aspirate pus using syringe, introducing as few bubbles as possible (think after to remove the needle and seal with a stopper).

Transport of the samples (put in syringe after removing the air / sterile container / no additive tube) to the laboratory must be carried out at room temperature within <2 hours.

9.5.12 Cerebrospinal fluid (not collected at IPC)

- Use dry tube.
- Transport to the laboratory at room temperature, immediately, without delay.

9.5.13 Superficial Pus

- Superficial pus: must be carried out on 2 gel transport swabs.
- Transport quickly (time <2 hours) at room temperature.
- Identify the swabs with the surname, first name, date, time and the sampling site.

9.5.14 Various biopsies (not collected at IPC)

- Place the biopsy in a sterile tube and possibly add (small samples) a few drops of sterile physiological water.
- Transport quickly (time < 2 hours) at room temperature.

9.5.15 Dermatophyte Research: Nails, Hair, skin

To be able to isolate the suspected dermatophyte, there must be a therapeutic window. A minimum period of time must be observed between stopping the antifungal treatment and sampling. All treatment must be stopped for at least:

- 3 months for systematic and/or local treatments with lacquer for nails or film-forming solution
- 15 days for an antifungal cream

Cleanse with soapy water in the shower in the morning, brushing the nails to remove the non-pathogenic fungi without using antiseptic soap.

Do not put moisturizer on the hands or feet the morning of the day of the sample, this interferes with direct examination.

Avoid nail polish.

If possible, clean the lesion with sterile water or saline solution.

If sampling at several sites, do not forget to indicate the sampling site on each vial or swab.

- Nails sampling:

Remove the infected part of the nail and throw it away. Collect dander by scratching at the junction between the affected area and the healthy area of the nail bed. Use vaccinostyle or curette; use sterile containers.

In case of oozing wound, collect purulent secretions with a swab.

- Skin sampling:

Collect dander in a sterile bottle (essential for direct examination) in periphery of the lesion by scraping (curette...)

If dander difficult to collect, a swab of the area scraped is possible for the culture.

Use vaccinostyle or curette; use sterile containers.

- Hair sampling:

  Recover affected hairs, dander and/or scabs scraping with a curette or tearing off with tweezers.

  Use sterile containers.

  Identify the samples with the surname, first name and the sampling site.

  Transport to the laboratory must be carried out at room temperature within 24 hours.

9.5.16 Specimen collection for Pityriasis versicolor (or Tinea versicolor)

The infection, caused by *Malassezia furfur*, interferes with the normal pigmentation of the skin, resulting in small, discolored patches. These patches may be lighter or darker in color than the surrounding skin and most commonly affect the trunk and shoulders.

The sample is carried out by Scotch-test after scraping the lesion.

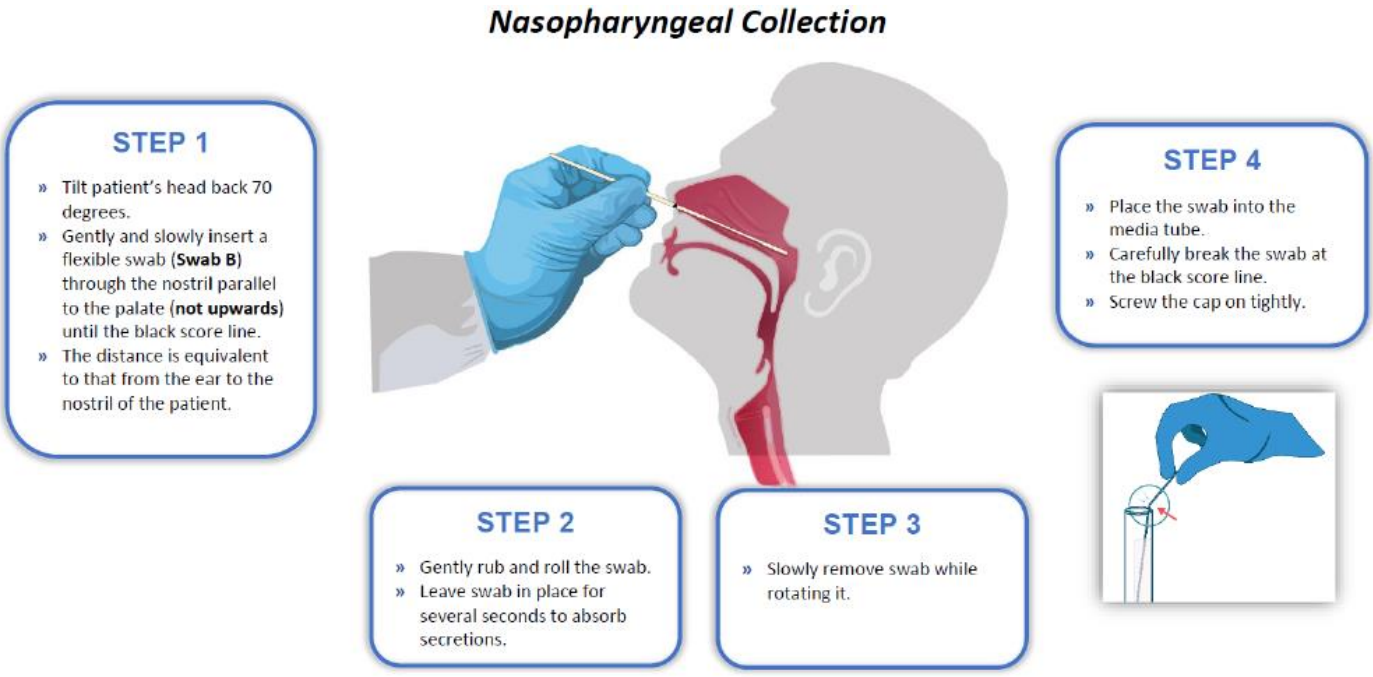
If the Scotch-test is not feasible on inflammatory or oozing lesions, take samples dander in a sterile vial for microscopic examination.

9.6 Nasopharyngeal swab for COVID-19 / Flu and RSV PCR

Use only synthetic fiber swabs with thin plastic or wire shafts that have been designed for sampling the nasopharyngeal mucosa.

Hygiene rules:

- Ensure that personal protective equipment (PPE) is worn properly.
- This includes gloves, a gown, eye protection and an N-95 or higher-level respirator.
- Gloves must be changed to a new pair for each patient, properly remove old pair and discard into a biohazard waster container.



For isolated detection of SARS-CoV-2, the transport to the laboratory must be carried out in transport media within 8h at room temperature or 7 days at 2-8°C.

For the combined detection of SARS-CoV-2/Flu/RSV, the transport to the laboratory must be carried out in transport media within 48h at room temperature or 7 days at 2-8°C.

Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.

9.7 Chlamydia/Gonorrhoea PCR

Several samples types are possible for the detection of Chlamydia and Gonorrhoea; if only molecular biology are requested (PCR test), without any bacteriological or mycological examination, following samples are recommended:

1) Men/Women: first catch urine sample

Special requirements: this test involves a "first pass" or "first catch" urine sample.

You must not have urinated within 1 to 2 hours before collecting your sample.

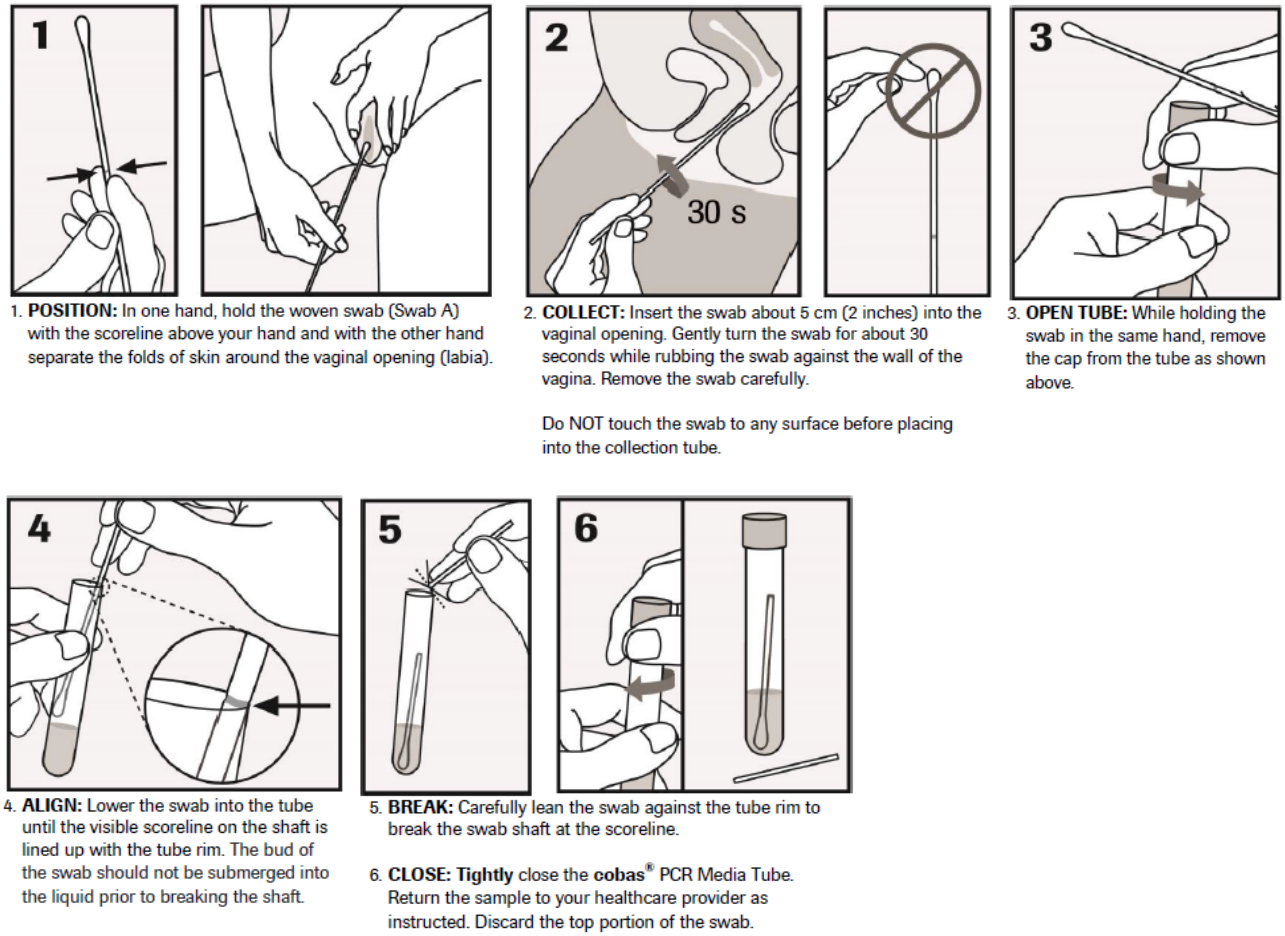
Catch the first 10 to 20ml of your urine flow. Do not exceed the recommended volume of urine to be collected as this may reduce the sensitivity of the test. The sample must be the "first part" of the urine stream.

- Wash your hands,
- Hold the container near your genital area,
- Commence urinating catching the first part of the urine (10-20ml),
- Once the 10-20ml is collected in the container, remove and continue to pass urine into the toilet,

- Secure the lid firmly,
- Transport of urine to the laboratory within 24h at room temperature (8 days at 2-8°C).

2) Women: endocervical (swabs provided by the laboratory on request)

3) Women: vaginal swab specimen-self-collection (swabs provided by the laboratory on request)



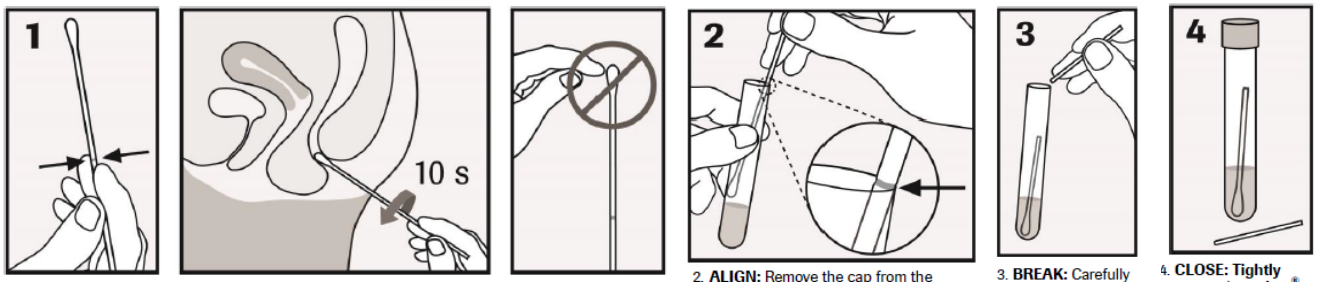
1. **POSITION:** In one hand, hold the woven swab (Swab A) with the scoreline above your hand and with the other hand separate the folds of skin around the vaginal opening (labia).
2. **COLLECT:** Insert the swab about 5 cm (2 inches) into the vaginal opening. Gently turn the swab for about 30 seconds while rubbing the swab against the wall of the vagina. Remove the swab carefully.

   Do NOT touch the swab to any surface before placing into the collection tube.
3. **OPEN TUBE:** While holding the swab in the same hand, remove the cap from the tube as shown above.
4. **ALIGN:** Lower the swab into the tube until the visible scoreline on the shaft is lined up with the tube rim. The bud of the swab should not be submerged into the liquid prior to breaking the shaft.
5. **BREAK:** Carefully lean the swab against the tube rim to break the swab shaft at the scoreline.
6. **CLOSE: Tightly** close the **cobas**® PCR Media Tube. Return the sample to your healthcare provider as instructed. Discard the top portion of the swab.

In case of prescription of both PCR and microbiological culture, please follow the instruction of vaginal and urethral samples and just add one more swab for the PCR test!

4) Rectal swab specimen collection (swabs provided by the laboratory on request)

The detection of Chlamydia/Gonorrhoea can be performed on anal swab depending on the sexual practices of the patient:



1. **COLLECT :** To collect the specimen, hold the swab with the scoreline above your hand and insert the swab about 3 to 5 cm into the anal canal. Gently turn the swab for about 5-10 seconds while running the swab against the walls of the rectum. If the swab is grossly contaminated with feces, discard and repeat the collection.

   Withdraw the swab carefully. Do not let the swab touch any surface before placing it into the collection tube.

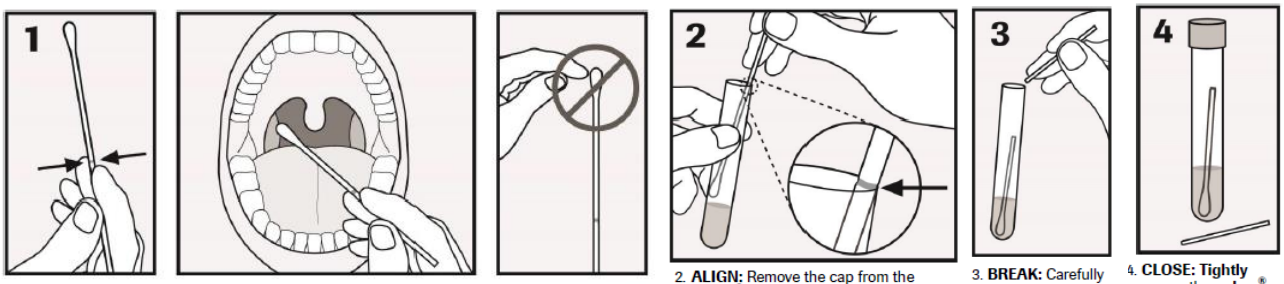
2. **ALIGN:** Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim. The tip of the swab should not be submerged into the liquid prior to breaking the shaft.

3. **BREAK:** Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.

4. **CLOSE: Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

5) <u>Throat swab specimen collection</u> (swabs provided by the laboratory on request)

The detection of Chlamydia/Gonorrhoea can be performed on throat swab depending on the sexual practices of the patient:



1. **COLLECT:** To collect the specimen, hold the swab with the scoreline above your hand and insert the swab into the mouth and collect the specimen from the bilaterial posterior pharynx, both tonsils and the uvula.

   Withdraw the swab carefully. Do not let the swab touch any surface before placing it into the collection tube.

2. **ALIGN:** Remove the cap from the **cobas**® PCR Media Tube and lower the swab specimen into the tube until the visible scoreline on the swab is aligned with the tube rim. The tip of the swab should not be submerged into the liquid prior to breaking the shaft.

3. **BREAK:** Carefully leverage the swab against the tube rim to break the swab shaft at the scoreline.

4. **CLOSE: Tightly** re-cap the **cobas**® PCR Media Tube. The specimen is now ready for transport. Discard the top portion of the swab.

After specimen collection, transport and store the sample at 2°C to 30°C. Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.

9.8 Human Papillomavirus (HPV) PCR

Obtain a sample from the cervix (endocervical specimen) with the **brush (see 9.5.8 Pap smear sample).**
Following specimen collection, transport and store the sample at 2°C to 30°C.
Transportation of collected specimens must comply with all applicable regulations for the transport of etiologic agents.

10. SAMPLE CONSERVATION AND TRANSPORT

Samples must arrive at Institut Pasteur during the opening hours of the Medical Biology Laboratory.
Many pre-analytical factors can affect the integrity of biological samples. Accordingly, these must be sent to MBL under conditions allowing compliance of the cold chain, according to the transport procedure described below.

10.1 Samples for biochemistry, serology, hematology, hemostasis

Samples must arrive at Institut Pasteur:

- For APTT (TCK): within 6 hours at room temperature after collection
- For Electrolytes (Na/K/Cl) in blood: within 4 hours after collection, if no centrifugation
- For Glucose in blood: within 24 hours after collection on the gray tube (fluoride), if no centrifugation
- For Lactic Acid: within 6 hours after collection on the gray tube (fluoride), if no centrifugation
- For LDH (Lactate Dehydrogenase): within 2 hours after collection, if no centrifugation
- For Phosphore in blood: within 6 hours after collection, if no centrifugation

Except for the analyses mentioned above, blood samples of biochemistry, hematology, hemostasis and immuno-serology can be stored for 24 hours before analysis.
For samples reaching the laboratory more than one day after collection, red and green tubes have to be centrifuged and decanted if possible. For decanted tubes, specify the specimen type on the form: plasma, serum.

10.2 Microbiology samples

Samples should be stored at +4°C before transportation, except for:

- Genital, Skin, Ear/Nose/Throat samples for bacteriology: must be kept at room temperature (must arrive at the MBL < 2 hours if no transport medium)
- Respiratory samples for bacteriology: must be kept at room temperature (must arrive at the MBL < 2 hours)
- Bone/Joint, Puncture fluid for bacteriology: must be kept at room temperature (must arrive at the MBL < 2 hours if no transport medium)
- Blood culture containers: must be kept at room temperature and sent as soon as possible to the laboratory
- CSF vials for meningitis research: must be kept at room temperature (must arrive at the MBL < 1 hour)

10.2.1 Urines

Urine is normally sterile, but unlike blood, it is an excellent culture medium in which contaminating germs will grow very quickly if the sample remains at room temperature and is not analysed quickly.
Therefore, the examination must be conducted within 2 hours after collection if it is stored at room temperature or < 12h if the urine is stored at 2-8°C.

IPC can provide BD Vacutainer preservative tube (Boric acid) upon request. The lyophilized urine maintenance formula can maintain the bacterial population in the urine specimen for up to 48 hours at room temperature at levels comparable to those without additives, held under refrigeration for the same time period.

10.2.2 Sputum

For standard sputum bacteriology, the examination must be carried out within 2 hours after collection and must be stored at room temperature.

For the diagnosis of tuberculosis, if the sputum cannot be transported to the laboratory on the day of collection, they must be kept under refrigeration:

- 48h for direct examination and culture
- 3 days at room temperature and 7 days under refrigeration for the Xpert MTB / RIF Ultra test.

10.2.3 Stool culture

Transportation delay < 2h at room temperature; otherwise, keep the stool maximum 12 hours at 2-8°C.

10.2.4 Other sample

Sample must arrive at the laboratory within 4 hours after collection, otherwise, keep at temperature 2-8°C.

10.3 Transport sampling

The triple packaging is used to carry out biological infectious products (Instruction 602_IATA - International Air Transport Association):

- The primary containers are tubes, vials, bottles, and swab holders (all waterproof). They must be labeled accordingly to the recommendations.
- The secondary containers are pouches with two compartments: one for the primary container and the second for the prescription.
- The last container is a rigid box, able to transport samples without damage.

11. RECEPTION

On arrival at the laboratory, the following elements are checked by the secretaries and the nurses:

- the conformity of the prescription,
- the concordance of the samples with the request,
- sample identification,
- compliance with the time before analysis,
- the required volumes (in particular, the correct filling of the tubes),

- the quality of the sample (hemolysis, lactescence, coagulation, etc.),
- the quality of the tube (expiration date),
- if necessary, the completed form with the required information (forms available on http://www.pasteur-kh.org)

Sample refusals include:

- unidentified and unidentifiable samples with certainty,
- citrate tubes not filled to the gauge mark,
- sample container not adapted to the requested test,
- samples on anticoagulants showing a clot,
- tubes that do not contain the minimum quantity of blood required to carry out the analyses,
- delivery times have been exceeded,
- expired tubes.

12. RESULTS

Availability of results:

Daily results are available from 4 pm, except in case of emergency.

The turn-around time of analysis is specified in the MBL Catalog.

By phone:

The results are never communicated by phone, except if alert value, in this case, the urgent or pathological results are communicated by the biologists, subject to biological validation.

By e-mail:

For prescribers and patients, it is possible to receive analysis reports by e-mail. In this case, please specify at the reception, a code will be communicated to you in order to access the result.

By results server:

Healthcare professionals can have access to the reports generated by our Laboratory Information System (LIS) via a results server.