# COLLECTION, STORAGE AND TRANSPORT OF SAMPLES

from field to reference laboratory



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Internal document 2022 edition

The Collection, storage and transport of samples from field to reference laboratory, 2<sup>nd</sup> edition (2022) has been developed by MSF, more specifically by the Laboratory working group.

MSF would like to express its sincere gratitude to the author-coordinator and to everyone who has contributed to developing this document

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# Introduction

These are internal, international MSF guidelines. They should make getting laboratory results for biological confirmation of epidemic-prone diseases faster and easier while complying with World Health Organisation (WHO) International Health Regulations (IHR). Only the WHO or a country's Ministry of Health can declare an epidemic.

These guidelines provide instructions on how to correctly collect and safely transport laboratory samples in order to detect pathogens capable of causing an epidemic. Samples must be collected, stored, and transported in a rigorous manner and monitored all the way to the reference lab. Regulations on the international transport of biological substances (dangerous substances) have become more stringent, and absolutely must be followed to avoid samples being held up at a border or refused when they arrive at the reference lab. It is important to fulfil all the criteria specific to each test requested and to fill out the test request correctly.

The reference lab will analyse the sample appropriately if given correct information about the patient and his disease.

Along with these guidelines, readers should have the most recent editions of the MSF Laboratory manual, Volume 5 of the MSF medical catalogue, and the Manual of Nursing Care Procedures. When readers need other MSF guidelines, the titles and references are given in the text.

These guidelines are divided into eight chapters:

#### 1. General information

- **2. Suspected disease/outbreak:** provides key information on which the samples to collect and which analyses should be performed in order to identify the causative organism for each disease.
- **3. Sample collection techniques:** provides a detailed description of sample collection procedures, along with detailed lists of the standard MSF equipment needed for those procedures.
- **4. Hygiene and safety:** review the safety and hygiene principles applicable to the sample collection and laboratory waste management procedures.
- **5. Lab test request forms:** these vary depending on the epidemic or suspected disease. They must accompany the samples to the lab. They should be used when the reference laboratory's own forms are unavailable.
- **6. National and international transport procedures:** these are fairly straightforward for category B UN3373 samples shipped via a carrier (DHL, for example), but more complicated for category A UN2814 "infectious substances" samples. Information about cold chain shipments is also available.

- **7. Test shipment registers:** provides one sample register for the field (where samples are collected) so the teams can track shipments and report results, and another that the medical coordination team can use for monitoring.
- **8. Laboratory contact information:** problems with exporting samples to MSF-chosen reference labs are increasing. There are only two identified international laboratories, and their contact information is provided in Chapter 8. Each mission should add the contact information for national reference labs or WHO labs available in the region. See Chapter 8.

Sample transport is constantly changing, and so this guide will need regular updating; comments and criticism are welcome.

Comments can be sent to: diagnostic-network@msf.org.

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# **Acronyms and abbreviations**

Ab Antibody Ag Antigen

ALAT Alanine aminotransferase

APU Amsterdam procurement unit

AWB Air waybill

CCHF Crimean-Congo haemorrhagic fever

CDC Center for Disease Control and Prevention

COPD Chronic obstructive pulmonary disease

COVID Coronavirus disease
CSF Cerebrospinal fluid
DBS Dried blood spot

DGD Dangerous goods declaration

EDTA Ethylenediaminetetraacetic acid

EIA Enzyme immunoassay

ELISA Enzyme-linked immunosorbent assay

ENT Ear, nose, and throat specialist

HEV Hepatitis E virus

IATA International Air Transport Association

IgG Immunoglobulin G
IgM Immunoglobulin M

IHR International health regulations

INSP Institut national de santé publique = National institute of public health

LP Lumbar puncture

MAT Microscopic agglutination test

MSF Médecins Sans Frontières

MTA Material transfer agreement

N/A Not applicable

NRC National Reference Centre

OCA Operational center Amsterdam

OCB Operational center Brussels

OCBA Operational center Barcelona

OCG Operational center Geneva

OCP Operational center Paris

PCR Polymerase chain reaction

PPE Personal protective equipment

RDT Rapid diagnostic test

RPM Revolutions per minute

RT-PCR Reverse transcription polymerase chain reaction

RVF Rift Valley fever

SOP Standard operating procedure TACT The air cargo tariff and rules

TI Trans-isolate

UIN Unique identification number

VHF Viral haemorrhagic fever

VTM Viral transport medium

# **Chapter 1: General information**

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# 1.1 General information

#### Lab tests are requested for the following purposes:

- When an outbreak is suspected:
  - To identify the causative agent responsible for the outbreak.
  - To identify the strains and/or serotype and/or genotype of the pathogen, if necessary.
  - To obtain a drug susceptibility test, if needed.
  - To monitor an ongoing outbreak and/or identify the final cases.
- When diagnosing a patient
  - To confirm a clinical diagnosis.
  - To detect the appearance of an emerging disease.

#### Each project should prepare for test requests and define each person's responsibilities.

#### Pre-positioning collection materials and protective equipment

Equipment for sample collection, sample transport, and personal protection must always be available, consistent with the risks of epidemic-prone infectious diseases identified in each country.

#### Accessible laboratory network

The exportation of lab samples is becoming increasingly difficult. The medical coordination team (with help from the laboratory supervisor and/or headquarters laboratory advisor) is responsible for assembling a list of labs in their country capable of performing the requested analyses. It is essential that the choice of laboratories be discussed with managers at headquarters (cell, medical department). The medical coordination team is also responsible for assessing both the types of tests available at the identified labs and MSF's capacity for sample collection, storage, and shipment before a potential outbreak.

If there are no labs that meet MSF requirements in the country, or if the requested tests cannot be done in-country, an attempt must be made to send them to an MSF-validated regional or international reference lab, with the national authorities' consent. In some cases, a duplicate shipment (one to the lab chosen by MSF and one to the lab recommended by the national disease surveillance system) will make it possible to ship the sample to a lab meeting MSF requirements; this should be discussed with the authorities.

The methods and information presented in these guidelines came from certain reference laboratories (see the list in Chapter 8): List of Laboratories and Contacts). Not all labs do the same types of analyses or use the same methods, however, so it is essential to contact the labs that MSF works with before sending samples to verify which ones are accepted and which analyses are done.

#### Material transfer agreement (MTA)

In accordance with MSF recommendations, no MTA is needed for outbreak investigations. Once the outbreak is confirmed, a framework agreement must be signed between MSF and the reference laboratory for the samples that are sent. In addition, in contexts where outbreaks

are known to recur (e.g., meningitis or cholera), a context-specific framework agreement must be signed with the labs in question (international or national). Contact the legal department for verification and approval of the context-specific framework agreement.

#### Verbal consent

Verbal consent is an unsigned agreement between the patient and the medical staff. Information such as the type of sample, the reasons for collecting it, where it will be analysed, and what the results will be used for is given to the patient verbally. The patient's decision is documented in his file.

For minors, consent must be given by a parent or guardian, using the usual procedure. Verbal consent should be documented in the patient's file by the physician/person in charge.

#### Protecting patient data



A unique identification number (UIN) should be used in place of the patient's first and last names.

The patient's name and UIN should only appear in the register kept in the laboratory at the sample collection location. If the UIN is unavailable, the physician should send the necessary information in a way that preserves confidentiality, so that the results are directed to the correct patient.

The principle is to maintain the right balance in terms of sending just enough information with the samples to ensure that the test/analysis results are redirected to the same patient.

#### Shipment notification

Always notify the capital medical coordination team, the cell, and the medical department of sample shipments.

The top priority is responsiveness and getting a result as quickly as possible.

After discussing with headquarters, notify the local authorities and Ministry of Health. In addition, it is necessary to contact the reference lab network (the WHO Collaborating Center (WHOCC), the CDC<sup>a</sup>, the Institut Pasteur, research laboratories, etc.) as quickly as possible to confirm certain information (sample storage duration and conditions) and coordinate the shipment. This allows proper transmission of information, confirmation of the sample type to be collected, and fast, appropriate handling (for example, it helps the reference lab prepare for the analyses).

#### Sample labelling, data collection, and follow-up

All samples must be labelled and accompanied by a test request form (see Chapter 5), including a clinical and epidemiological description. Without those documents, the laboratories that receive the samples may refuse to perform the analyses.

The source of each sample must be clearly identified with the name of the person responsible for the shipment and the name of the person to whom the results should be sent.

a CDC: Centers for Disease Control and Prevention

All samples will be logged in two types of registers:

- ✓ A field register, which contains information about the patient and the sample, including the results.
- ✓ A capital register, which contains information about the sample and the shipment, but no patient names.

In the field, the register should be completed by the laboratory manager. In the capital, it should be completed by the medical coordination team with help from the logistics coordination team.

#### Regulations for sample shipments

Transporting samples in baggage when travelling from the field is strictly prohibited. Whether shipped nationally or internationally, UN3373 and UN2814 samples must be shipped in triple packaging boxes. All the details are available in Chapter 6.

The logistics coordination team is responsible for knowing the regulations in each country and the shipment options for UN3373 (Infectious Substance, Category B) and UN2814 (Infectious Substance, Category A) samples. Note that this information can change over time:

- ✓ Advance contacts with DHL for UN3373 sample shipments.
- ✓ List of airlines or other authorised carriers available locally that will transport UN2814 samples. This list should be reviewed at least once a year.

#### • Responsibility matrix: field, capital, and headquarters

**Table 1** - Roles and responsibilities in the MSF team when an epidemic is being prepared for, suspected, or confirmed.

	Field	Capital	Headquarters
In pr	reparation for a poss	ible epidemic	
List and visit the reference labs (evaluate possible analyses and methods used).	Laboratory man- ager/ supervisor	Medical coordination	Laboratory Advisor
Contact the department respon-sible at the national disease sur-veillance system (e.g., measles, polio, meningitis, etc.)		Medical coordination	
Contact the carriers (DHL) for Category B - UN3373 shipments		Logistics coordination	
List and contact the airlines or carriers for Category A - UN2814 shipments annually		Logistics coordination	

	Field	Capital	Headquarters				
If an outbreak is suspected							
Notify the MSF coordination team and the cell.	Medical officer (notifies the co- ordination team)	Medical coordination (notifies the cell)					
Notify the Ministry of Health and local authorities		Medical coordination					
Contact the reference lab		Medical coordination if the lab in is the country.	Laboratory advisor or headquarters medical department if the lab is outside the country.				
Contact the carriers/airlines for sample shipments		Logistics coordination					
Obtain a "Sample export permit" from the Ministry of Health or relevant authority.		Medical coordination					
For international shipments, email the shipping documents to the reference lab.		Medical coordination					
Complete the sample tracking registers.	Laboratory man- ager or medical officer	Medical (and logis-tics) coordination					
Receive and follow-up on the results.		Medical coordination	Medical Department				
After confirmation of an epidemic (by the WHO or Ministry of Health)							
Get a signed MTA if samples are sent to an outside lab.		Medical coordination team	Medical and legal departments				



The declaration of an epidemic falls to the Ministry of Health and the WHO.

#### Methods for demonstrating the presence of pathogens

- Direct methods for demonstrating the presence of the causative disease agent or its components:
  - By culturing and/or isolating the disease causative agent
  - By detecting some component of the pathogen: antigen, genetic material, etc.: rapid diagnostic test (RDT), gene amplification (PCR, RT-PCR), etc.
- Indirect methods that detect a host immune response specific to the disease causative agent (presence of antibodies): serological tests (ELISA, EIA, RDT, etc.)

The laboratory testing strategies for diagnosing a patient and for investigating an outbreak are different and should always consider the epidemiological context (endemic area or not). Consult with the national disease surveillance department and refer to the established national protocols.

This guide lists only those samples most appropriate to the field conditions. The most commonly used analyses are indicated in bold type. Some experimental methods are also given, for information purposes, in the second chapter. The analyses might use direct or indirect methods, depending on the reference laboratory, and sometimes a combination of the two.

#### Interpretation of antibody results

Viruses, bacteria, parasites, and fungi all elicit the classic immune response with IgM, followed by IgA and IgG. The presence of IgM indicates a recent infection. To find antibodies in the blood, collect a sample 5 to 8 days after the first symptoms appear. With many viral infections, it may take eight days to three weeks for IgM to appear, while for practical reasons the sample is often taken at admission. This time frame for seroconversion must be taken into account when interpreting results. The presence of IgG does not, in and of itself, indicate ongoing infection. It might be due to past infection or vaccination. To demonstrate a seroconversion in progress, the antibody titre must increase between two samples. The first sample should be taken at admission, and the second two to three weeks later (a second sample collected too early is useless). In practice, if only one sample is possible, the time frame for seroconversion must be taken into account when interpreting results

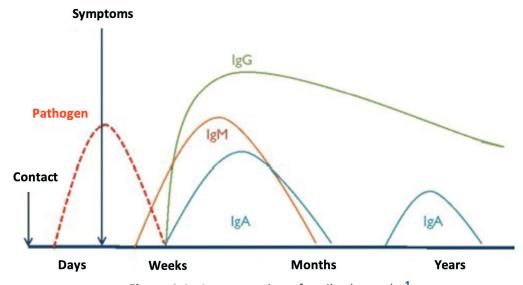


Figure 1.1 - Interpretation of antibody results<sup>1</sup>

In addition, for some diseases (arboviroses), cross-reactivity can complicate interpretation of the results.

# **References Chapter 1**

1. Reto Lienhard. « Pièges en sérologie infectieuse », Revue médicale suisse, 2011, 312. (Avec autorisation de reproduction).

https://www.revmed.ch/revue-medicale-suisse/2011/revue-medicale-suisse-312/pieges-en-serologie-infectieuse

# **Chapter 2: Suspected disease/outbreak**

# **Viral diseases**

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# 2.1 Arboviroses

#### **2.1.1** Dengue

When the clinical picture suggests a haemorrhagic fever, see Chapter 2, Section 2.3.

#### The disease

Dengue is an arbovirus (*Flaviviridae* family) transmitted to humans via the bite of a mosquito from the genus *Aedes*. The dengue virus has four serotypes, numbered 1 to 4.

The disease usually presents as a flu-like syndrome. Most infected individuals recover without treatment, but a small percentage will progress to the severe, potentially fatal, haemorrhagic form (thrombocytopenia). It presents in three phases: the febrile phase (which can last from 2 to 7 days), the critical phase (day 3 to day 7), and the convalescent phase (which begins on day 8).

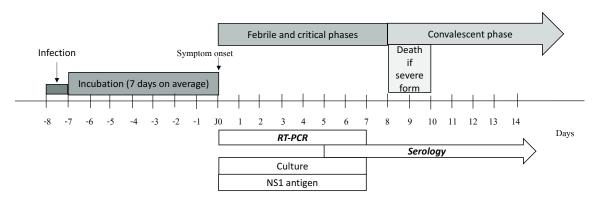


Figure 2.1 - Clinical course of primary dengue and timing of sample collection

#### **Available methods**

- In the field: there is a rapid diagnostic test (RDT) that detects both NS1 antigen (detectable during the febrile phase) and IgG and IgM antibodies, which can be detected during the critical and convalescent phases. This test should not be used for diagnosing patients suspected of having dengue, but only for outbreak investigations, due to the performance limitations of currently available tests. While a positive rapid test helps determine the extent of virus circulation, it cannot be used to determine the number of cases actually infected or declare an epidemic. Contact the operational centre laboratory advisor with any questions (to identify the appropriate algorithm for the context).
  - ELABTIME1E- TIMER, electronic
  - SSDTDENG10T DENGUE NS1/IgM/IgG TEST (Dengue Duo),ser/pl/wb,1 test 11FK45
  - + material for blood sample collection
- Direct techniques: RT-PCR, isolation in culture.
- Indirect techniques: serological tests/IgG and IgM detection (ELISA, serum virus neutralisation, or immunotitration).

Due to cross reactions between the different arboviruses, the reference laboratory may need to perform several concurrent analyses to confirm the clinical diagnosis.

### Samples to collect and collection and shipping conditions



The type and number of samples to be collected will depend on the reference laboratory. Check in advance.

**Table 2.1** - Samples to collect and collection and shipping conditions – Dengue

Test Type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collection and arrival at reference laboratory	Shipping temperature
RDT	Starting on Day 1 and throughout the course of the disease	Whole venous blood (100 microliters for NS1 Ag and 10 microliters for IgG/IgM Ac) or serum or plasma	N/A	N/A	N/A	N/A
		Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
DT_DCD	Within 7 days of symptom onset	At least 2 DBS (venous or capillary blood)	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
Starting Day 5	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C	
Serology	after symptom onset	At least 2 DBS (venous or capillary blood)	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C à +8 °C
Viral culture	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8° C

Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).

- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, Section 3.2.1.
  - Preparing serum and plasma, Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, Section 3.2.4.
  - Collecting a CSF sample Section 3.10.1 and transferring CSF into a cryotube Section 3.10.3.
  - Collecting a urine sample Section 3.7.

### Test request form and logging

See the Arbovirus test request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected dengue virus infection are Category B and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.1.2 Yellow fever



When the clinical picture suggests a haemorrhagic fever, see Chapter 2, Section 2.3.

#### The disease

Yellow fever is an arbovirus (*Flaviviridae* family) transmitted to humans primarily via the bite of a mosquito from the genus *Aedes*, and to a lesser extent from the genus Haemagogus. In 15% of cases it causes acute haemorrhagic disease and/or jaundice. The case fatality rate can be as high as 20% to 50% of cases<sup>1</sup>.

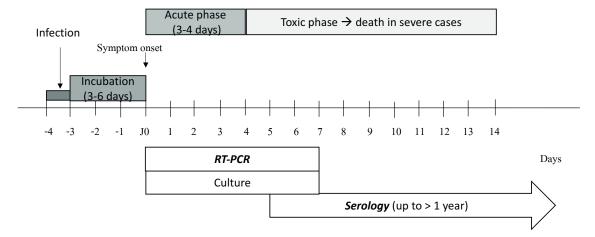


Figure 2.2 - Clinical course of yellow fever and timing of sample collection

#### **Available methods**

- Direct techniques: **RT-PCR**, isolation in culture.
- Indirect techniques: serological tests/IgG and IgM detection (ELISA, serum virus neutralisation, or immunotitration):
  - Presence of IgM antibody, or
  - Elevated anti-yellow fever IgM or IgG titres in paired serum samples (acute and convalescent phase), or
  - Demonstration of yellow fever-specific neutralising antibodies (results of testing for other flavivirus-type antibodies are negative or not significant), and
  - No yellow fever vaccination in the preceding 30 days

Due to cross reactions between the different arboviruses, the reference laboratory may have to perform several concurrent analyses to confirm the clinical diagnosis

#### Sample type and amount, timing of collection, and storage



The type and number of samples to collect will depend on the reference laboratory. Check in advance.

Table 2.2 - Samples to collect and collection and shipping conditions – Yellow Fever

Test Type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collection and arrival at reference laboratory	Shipping temperature	
		Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C	
RT-PCR Within 7 days of symptom onset	At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C		
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C	
			Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
Serology Starting Day 5 after symptom onset	Starting Day E	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection.	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C	
	Minimum 2 spots DBS (sang veineux)	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C		
		CSF: 0.5 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C	
Viral culture	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C	
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C	
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C	

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.
  - Collecting a urine sample, see Section 3.7.

#### Test request form and logging

See the Arbovirus test request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected yellow fever infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.1.3 Japanese encephalitis

#### The disease

Japanese encephalitis is an arbovirus (*Flaviviridae* family) transmitted to humans via the bite of a mosquito from the genus *Culex*. While most infections are asymptomatic, encephalitis occurs in an estimated one out of every 250 infections<sup>2</sup>. The case fatality rate can reach 30% when that happens, and 30 to 50% of survivors suffer neurological sequelae<sup>3</sup>.

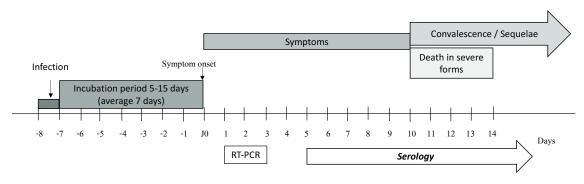


Figure 2.3 - Clinical course of Japanese encephalitis and timing of sample collection

#### **Available methods**

- Indirect techniques: serological tests IgM-capture ELISA, usually in CSF and plasma or serum.
- Direct techniques: RT-PCR. However, given that virus levels in the blood are low and short-lived (24 to 72 hours) at symptom onset, these tests are generally not informative.

Due to cross reactions between the different arboviruses, the reference laboratory may have to perform several concurrent analyses to confirm the clinical diagnosis.

#### Sample type and amount, timing of collection, and storage



The type and number of samples to collect will depend on the reference laboratory. Check in advance.

Table 2.3 - Samples to collect and collection and shipping conditions — Japanese Encephalitis

Test type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collec-tion and arrival at reference laboratory	Shipping temperature
Serology	Starting Day 5 after symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection.	+2 °C to +8 °C	2 weeks (ideally: 1 week)	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C
		CSF: 10 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C
RT-PCR	Between 24 hrs. and 72 hrs. after symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection.	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C
		CSF: 0,5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine : 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- **SOP:** see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.
  - Collecting a urine sample, see Section 3.7.

#### Test request form and logging

See Arbovirustest request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment. Sample information must be entered in the registers.

#### **Transport**

Samples for suspected Japanese encephalitis infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.1.4 West Nile fever

#### The disease

West Nile fever is an arbovirus (*Flaviviridae* family) transmitted to humans via the bite of a mosquito, primarily those from the genus *Culex*.

In less than 1%<sup>4</sup> of those infected, West Nile fever can cause neuroinvasive disease – encephalitis, meningitis, and even a poliomyelitis-type acute flaccid paralysis – which can be fatal.

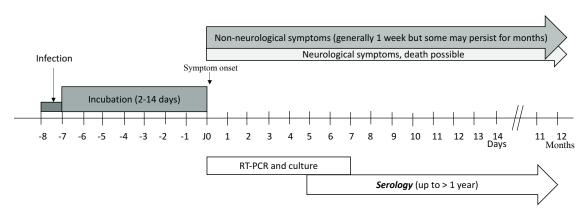


Figure 2.4- Clinical course of West Nile fever and timing of sample collection

#### **Available methods**

- Indirect techniques: serology (ELISA and serum virus neutralisation).
- Direct techniques: RT-PCR and viral culture. RT-PCR is more sensitive on CSF than on blood, but only for symptomatic patients

Two samples are needed for interpretation of the serological analysis: one early sample and one late sample (taken 14-days after the first).

Due to cross reactions between the different arboviruses, the reference laboratory may have to has to perform several concurrent analyses to confirm the diagnosis.

#### Sample type and amount, timing of collection, and storage



The type and number of samples to collect will depend on the reference laboratory. Check in advance.

Table 2.4 - Samples to collect and collection and shipping conditions – West Nile fever

Test type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collec-tion and arrival at reference laboratory	Shipping temperature
Serology	Starting Day 5 after symp-tom onset, and then 14 days after the first sample is collected	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C
RT-PCR	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
Viral culture	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.
  - Collecting a urine sample, see Section 3.7.

#### Test request form and logging

See Arbovirustest request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment. Sample information must be entered in the registers.

#### **Transport**

Samples for suspected West Nile virus infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.1.5 Zika

#### The disease

Zika is an arbovirus (*Flaviviridae* family) transmitted to humans via the bite of a mosquito from the genus *Aedes*. It can also be transmitted sexually, through breastfeeding, and via blood transfusion.

The disease is often asymptomatic and mild, lasts about one week. Zika virus infection can cause symptoms like fever, skin rash (exanthem), conjunctivitis, muscle and joint pain, malaise, and headache. If the infection is contracted during pregnancy it can be transmitted to the foetus and may cause a severe malformation, microcephaly, resulting in irreversible intellectual disability.

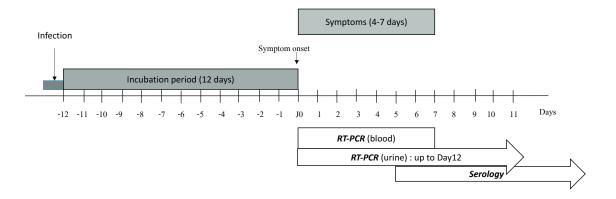


Figure 2.5 - Évolution clinique du Zika et chronologie de prélèvements

#### **Available methods**

- Direct techniques: RT-PCR and viral culture.
- Indirect techniques: **serology-IgM levels** (ELISA and serum virus neutralisation).

Due to cross reactions between the different arboviruses, the reference laboratory may have to perform several concurrent analyses to confirm the diagnosis.

#### Sample type and amount, timing of collection, and storage



The type and number of samples to collect will depend on the reference laboratory. Check in advance.

**Table 2.5** - Samples to collect and collection and shipping conditions — Zika

Test type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collec-tion and arrival at reference laboratory	Shipping temperature
RT-PCR	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °Cto +8 °C	1 week	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
Serology	Starting Day 5 after symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection	+2 °C to +8 °C	2 weeks (ideally 1 week)	+2 °C to +8 °C
		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C
Viral culture	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
Salture		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 weeke	+2 °C to +8 °CC
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.
  - Collecting a urine sample, see Section 3.7.

#### Test request form and logging

See Arbovirustest request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment. Sample information must be entered in the registers.

#### **Transport**

Samples for suspected Zika infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.1.6 Chikungunya

#### The disease

Chikungunya is an arbovirus (*Togaviridae* family) transmitted to humans via the bite of a mosquito from the genus *Aedes*.

It causes a sudden-onset fever and severe, often disabling joint pain that generally resolves within a few days or weeks. In some cases, the joint pain can last several months or even years.

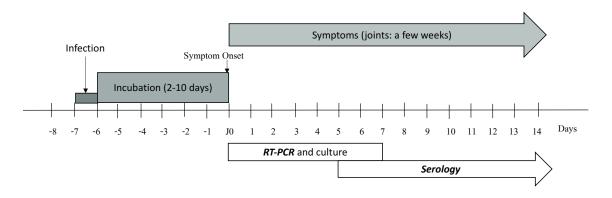


Figure 2.6 - Clinical course of chikungunya and timing of sample collection

#### **Available methods**

- Direct techniques: RT-PCR and viral culture.
- Indirect techniques: **serology-IgM levels** (ELISA and serum virus neutralisation).

Due to cross reactions between the different arboviruses, the reference laboratory may have to perform several concurrent analyses to confirm the diagnosis.

#### Sample type and amount, timing of collection, and storage



The type and number of samples to collect will depend on the reference laboratory. Check in advance.

**Tableau 2.6** - Samples to collect and collection and shipping conditions — Chikungunya

Test type	Timing of sample collection	Sample type	Time before centrifugation and separation	Sample storage after separation	Maximum time between collec-tion and arrival at reference laboratory	Shipping temperature
	RT-PCR Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
RT-PCR		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8 °C or <25 °C protected from light and moisture	1 week	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
	Starting Day 5 after symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 24 hours of collection	+2 °C to +8 °C	2 weeks (ideally 1 week)	+2 °C to +8 °C
Serology		At least 2 DBS – venous blood	Dry DBS (3-4 hrs. of drying)	Ideal: +2 °C to +8° C or <25 °C protected from light and moisture	2 weeks	+15 °C to +25 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	2 weeks	+2 °C to +8 °C
Viral culture	Within 7 days of symptom onset	Serum: 2 mL or Plasma: 2 mL	Separate the serum or plasma within 2 hours of collection	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		CSF: 0.5 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C
		Urine: 1 mL	NA	+2 °C to +8 °C	1 week	+2 °C to +8 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.
  - Collecting a urine sample, see Section 3.7.

#### Test request form and logging

See Arbovirustest request form (Chapter 5, Section 5.1). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment. Sample information must be entered in the registers.

#### **Transport**

Samples for suspected Chikungunya virus infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

### 2.2 COVID-19

#### The disease

COVID-19 is caused by the SARS-CoV-2 virus (*Coronaviridae* family), responsible for the global pandemic declared by the WHO on 11 March 2020.

In most cases, the disease is asymptomatic or paucisymptomatic with flu-like symptoms. The most common symptoms are fever, a dry cough, and fatigue. It can cause pneumonia with respiratory distress in at-risk patients (obesity, diabetes, hypertension, and chronic lung disease).

According to what is currently known (as of early 2021), the disease seems to have three phases: an incubation phase that lasts an average of 5 days after initial contact; a symptomatic phase that starts around Day 5; and, in some cases, a phase in which respiratory symptoms worsen around Day 7 or 8, potentially leading to death in some patients. This information should be updated as knowledge changes.

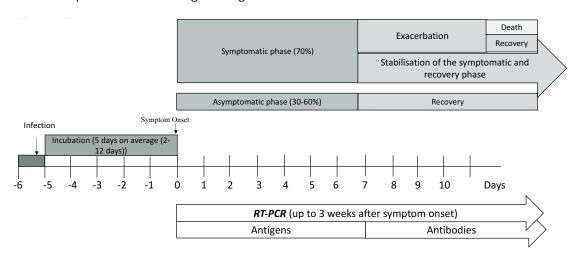


Figure 2.7 - Clinical course of COVID-19 and timing of sample collection

#### **Available methods**

- Direct technique (gold standard): RT-PCR.
- There are rapid tests, called antigen tests, which identify viral proteins. Those tests appear
  to detect high viral loads. They should be the first-line test, done immediately on the patient,
  as the antigens do not last long.
- There are also tests that detect IgM/IgG antibodies, which can be used to assess exposure
  to the disease. They cannot be used to diagnose the disease. Those tests are not currently
  recommended by the WHO to diagnose acute infection. For the latest on tests and their
  indications, consult your operational section laboratory advisor.

#### Sample type and amount, timing of collection, and storage

Collect a nasopharyngeal swab and an oropharyngeal swab and send them to the laboratory in the same transport medium tube. If, however, there is only one swab available, take a nasopharyngeal sample (higher viral load).

Specifics regarding swabs and transport medium:

#### Swabs

- Swabs should have a polyester/rayon/nylon tip, ideally flocked (no cotton)
- Plastic shaft (not wooden)
- Sterile
- Two swabs per patient (one with a mini-tip for the nasopharyngeal sample, the other with a normal tip for the oropharyngeal sample)

#### Viral transport medium (1 per patient)

- 1, 2, or 3 mL of isotonic viral transport medium isotonic to mammalian host cells, with antibiotics to inhibit the growth of concurrent bacteria and yeasts.
- Cannot contain guanidine thiocyanate (= toxic).

The swabs and transport media should meet the CLSI M40-A standard.

Table 2.7 - Samples to collect and collection and shipping conditions – COVID-19

	Sample type	Timing of sample collection	Storage temperature	Time between collection and arrival at the lab	Shipping temperature
RT-PCR	Nasopharyngeal swab Oropharyngeal swab	As soon as symptoms appear or 7 days after a known contact	+2 °C to +8 °C	12 days, maximum	+2 °C to +8 °C

The WHO has issued intermediate recommendations on the potential role of rapid antigen tests for diagnosing COVID-19, and on the need to be careful when choosing such tests (see the latest recommendations at, https://www.who.int/fr/emergencies/diseases/novel-coronavirus-2019/technical-guidance).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a nasopharyngeal swab, see Section 3.5.2.
  - Collecting an oropharyngeal swab, see Section 3.5.3.

#### Test request form and logging

See the COVID-19 test request form (Chapter 5, Section 5.2). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected COVID-19 infection are Category B samples and are labelled UN3373 (See Chapter 6, Section 6.3 for more details).

## 2.3 Viral haemorrhagic fevers

Viral haemorrhagic fevers (VHF) are highly contagious and require full personal protective equipment when collecting all samples (see Chapter 4 for complete recommendations).

#### The diseases

These are serious diseases caused by different viruses, and are often accompanied by haemorrhagic signs, in particular: viruses from the *Arenaviridae* family (Lassa fever, Argentine haemorrhagic fever, and Bolivian haemorrhagic fever); the *Bunyaviridae* family (Crimean-Congo haemorrhagic fever, Rift Valley fever, and Hantavirus haemorrhagic fever with renal syndrome); the Filoviridae family (Ebola and Marburg); and the *Filoviridae* family (yellow fever, dengue, Omsk haemorrhagic fever, and Kyasanur forest disease).

Some are arbovirus transmitted by mosquitoes or ticks (Crimean-Congo haemorrhagic fever, Rift Valley fever, yellow fever, and dengue). Others are transmitted by rodents (Lassa fever), by contact with monkey meat (bush meat), or by bats (Ebola and Marburg). The following VHFs carry a risk of human-to-human transmission: Ebola, Marburg, Lassa fever, and Crimean-Congo haemorrhagic fever.

#### 2.3.1 Lassa fever

Lassa fever is asymptomatic in 80% of cases. In 20% of cases it begins gradually and can involve multiple organs (kidney, spleen, and liver). The case fatality rate is low, at about 1%<sup>5</sup>. When infection occurs during the third trimester of pregnancy, there is a risk of foetal death in 80% of cases and a significant risk of maternal death. The disease generally lasts one to four weeks, and is transmitted via contact with the excreta of rodents (which are the reservoir), via contaminated medical equipment, or by human-to-human transmission (body fluids). Lassa fever can cause small-scale nosocomial outbreaks.

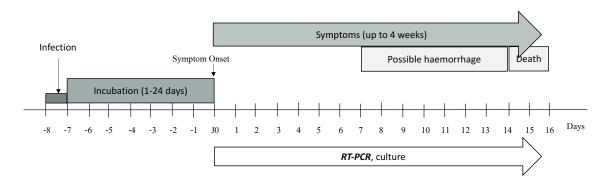


Figure 2.8 - Clinical course of Lassa fever and timing of sample collection

#### 2.3.2 Filovirus: Ebola and Marburg virus disease

#### The disease

Ebola and Marburg are serious diseases that are very often fatal (the case fatality rate can be as high as 90% for Ebola<sup>6</sup> and 88% for Marburg<sup>7</sup>). They are transmitted to humans via contact when handling infected bush meat or the excreta of bats (which are the reservoir). Human-to-human transmission occurs via direct contact with the body fluids of symptomatic patients or via contact with soiled surfaces or equipment. With Ebola virus disease, after a 2- to 21-day incubation period the patient develops fever and fatigue that can progress to internal bleeding and/or external bleeding in some cases. Patients become contagious as soon as the initial symptoms appear.

With Marburg virus disease, the incubation period lasts 2 to 21 days and disease onset is sudden, with high fever and severe malaise. Severe bleeding can develop after 5 to 7 days, leading to death.

The Ebola and Marburg viruses persist in body fluids (urine, placenta, amniotic fluid, breast milk, sperm, etc.) for several weeks or months after the patient recovers. The corpses of patients infected with these pathogens remain contagious after death.

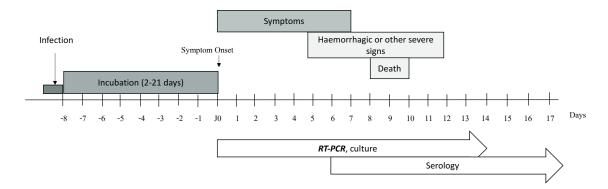


Figure 2.9 - Clinical course of Ebola and Marburg virus disease and timing of sample collection

#### 2.3.3 Rift Valley fever

#### The disease

Rift Valley fever (RVF) is a viral zoonosis (*Bunyaviridae* family) that can be transmitted to humans either by the bite of a mosquito (genus *Aedes* and in some cases *Culex*) or by exposure to blood (aerosolised during animal slaughter, or from injuries), to body fluids, or to the tissues of infected animals.

The infection can be severe in a small percentage of patients (fewer than 2% have the ocular form, fewer than 1% have the meningoencephalitis form, and fewer than 1% have the haemorrhagic form). The overall case fatality rate is low (less than 1)<sup>8</sup>.

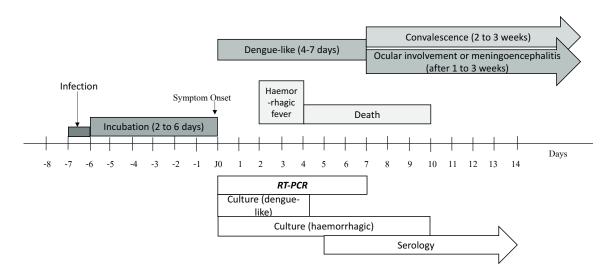
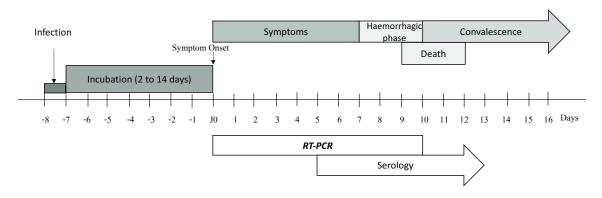


Figure 2.10 - Clinical course of Rift Valley fever and timing of sample collection

#### 2.3.4 Crimean-Congo haemorrhagic fever

#### **Pathologie**

Crimean-Congo haemorrhagic fever (CCHF) is caused by a virus from the *Bunyaviridae* family. It is transmitted to humans via tick bite or contact with tissues or blood from an animal infected by ticks. The disease can cause small-scale nosocomial outbreaks with case fatality rates ranging from 10 to 40%<sup>9</sup>. CCHF patients can develop massive bleeding due to disseminated intravascular coagulation, as well as respiratory failure, hepatorenal failure, and/or coma.



**Figure 2.11** - Clinical course of Crimean-Congo haemorrhagic fever and timing of sample collection

#### Available diagnostic methods for all VHFs

- EBOLA Zaire strain: GeneXpert® with the appropriate cartridge
- All viral haemorrhagic fevers: standard PCR techniques –(non-commercial kits often used when there are no validated commercial kits available)
- Indirect techniques: ELISA IgM/IgG, and neutralization assay

#### Samples to collect and sample collection conditions

#### - Sample type and storage

**Table 2.8** - Samples to collect and collection and shipping conditions — Viral haemorrhagic fevers

Sample type	Patient	Transport medium	Storage temperature	Time frame for shipping to reference lab	Transport temperature
4 mL whole venous blood – non-haemolysed	Symptomatic patient	N/A	+2 °C to +8 °C	As soon as possible (up to one week)	+2 °C to +8 °C
Capillary blood  – as much as possible – non- haemolysed	If venous blood cannot be collected	N/A	+2 °C to +8 °C	48 to 72 hours	+2 °C to +8 °C
Buccal swab (EBOLA ONLY)	On a deceased patient or if blood draw impossible on	Viral transport medium	+15 °C to +25 °C	24 hours	+15 °C to +25 °C
·	a symptomatic patient		+2 °C to +8 °C	1 week	+2 °C to +8 °C

Whole venous blood: 4-mL EDTA/purple tube (filled to the indicated limit). The sample can be rejected if haemolysed.

Other samples (e.g., urine, CSF, serum, and biopsies) may be collected after consultation with the reference laboratory and may require special shipping conditions.

#### - Timing of sample collection

If possible, collect the sample while the patient is febrile.

The RT- PCR test may cause a false negative result if done early in the disease because of low viral load activity. Ebola and Marburg infections can only be ruled out if an RT-PCR test was done five or more days after symptom onset. If the sample was collected early in the disease, a second RT-PCR should be done (48 hours after the first sample was collected).

#### Sample collection procedure

 $\triangle$ 

When a haemorrhagic fever is suspected, it is essential to handle the sample as little as possible and follow the rules for personal protection.

Consult the technical advisor for further details.

#### Hygiene: see Chapter 4.

Personal protective equipment is required:

- Two pairs of gloves
- Gown/overgown or coverall
- Tie-back scrub cap or hood, FFP2/N95 mask, goggles
- Waterproof apron
- Rubber boots

After collecting the sample and undressing, wash gloved hands with a 0.5% chlorine solution and then wash hands with soap and water after removing both pairs of gloves.

Lab waste should first be chemically inactivated (using a 0.5 to 1% chlorine solution) and then placed into a first leak-proof container. That container should then be placed in a second container for transport to the treatment centre's incineration area.

Sharps used for collecting samples should be placed into sharps collectors, and the other waste into trash bags that are sprayed with 0.5% chlorine solution and then burned.

- SOP: see Chapter 3.
  - Collecting a venous blood sample for suspected viral haemorrhagic fever with a Vacutainer<sup>®</sup> system, see Section 3.2.2.
  - Buccal swab in viral transport medium, see Section 3.5.5.

#### Test request form and logging

See the test request form for VHFs (Chapter 5, Section 5.3). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

All samples for diagnosing suspected VHF (Ebola, Marburg, Lassa, Rift Valley fever, or Crimean-Congo haemorrhagic fever) are Category A and must be transported as infectious substances labelled UN2814 (see Chapter 6, Section 6.4 for more details).

#### **Reference laboratory**



It is imperative to contact the headquarters laboratory advisors before sending any samples.

## 2.4 Hepatitis E

#### The disease

Hepatitis E is a disease caused by four genotypes of the hepatitis E virus (HEV), from the Hepaviridae family. It is transmitted by the faecal-oral route and is responsible for outbreaks, especially in IDP or refugee camps. Severe forms with a clinical presentation of fulminant hepatitis (acute liver failure) can occur in 1% of cases, or in as many as 45% of cases among pregnant women<sup>10</sup>. The infection is most severe in the third trimester of pregnancy, with a mortality rate as high as 25%<sup>11,12,13</sup>.

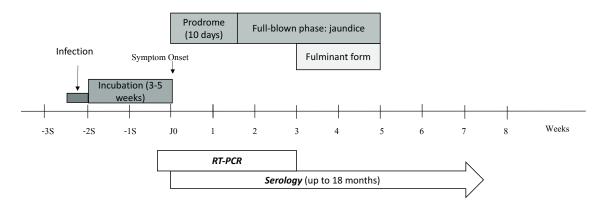


Figure 2.12 - Clinical course of hepatitis E and timing of sample collection.

#### **Available methods**

- In the field, a rapid diagnostic test that detects anti-HEV IgM antibodies can be used. It is a tool for outbreak investigation only, not for diagnosing individuals. MSF does not currently have a standard rapid diagnostic test for hepatitis E, due to the lack of information on test performance in the contexts where MSF works. A non-standard test may be ordered, but only after validation by the MSF operational centre medical director. Contact the laboratory advisor for more information.
- Direct technique: quantitative RT-PCR and genotyping
- Indirect technique: serology (ELISA): IgM detection

#### Samples to collect and sample collection and storage conditions

Check with the reference lab regarding the type of sample expected, the required storage conditions, and the maximum allowable time between sample collection and reception, according to the technique used.

<b>Table 2.9</b> - Samples to collect and collection and shipping conditions – Hepatitis	Table 2.9 - Samp	es to collect and	d collection and	shipping conditi	ons – Hepatitis E
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Analysis	Sample type	Timing of sample collection	Amount	Allowable time between collection and arrival at reference lab	Storage and shipping temperature
FLICA and	Serum	Symptomatic	1 mL	Ideally, less than a week	+15 °C to +25 °C
RT-PCR	ELISA and RT-PCR DBS with capillary or venous blood	patient (high viral load during jaundice)	5 spots	No specific time	+15 °C to +25 °C
DT DCD	Stool in a non- sterile sample container		Walnut size	No specific time	+15 °C to +25 °C
RT-PCR	Stool in viral transport medium		1 swab	No specific time	+ 15 °C to +25 °C

- Serum: use a 4-mL dry/red Vacutainer<sup>®</sup> tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

Hepatitis E virus is very robust and can tolerate being stored at ambient temperature for several days.

#### Sample collection procedure

- Hygiene: see Chapter 4.
- **SOP:** see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum, see Section 3.3.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.
  - Collecting a stool sample, see Section 3.6.
  - Faecal swab in viral transport medium, see Section 3.6.2.

#### Test request form and logging

See the Hepatitis E test request form, Chapter 5, Section 5.4. This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected hepatitis E infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

## 2.5 Poliomyelitis



Poliomyelitis is a notifiable disease; report to the Ministry of Health and/or the WHO.

#### The disease

Poliomyelitis is an acute, highly contagious viral infection caused by the poliovirus (serotypes 1, 2, and 3); it mainly affects children. It is transmitted primarily via the faecal-oral route. The virus invades the nervous system. The disease is asymptomatic in 90% of cases and in the other 10% it can cause symptoms in a few hours, progressing in 1% to 2% of cases to an asymmetric flaccid paralysis, mainly in the lower extremities <sup>14</sup>.

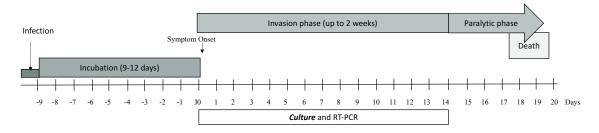


Figure 2.13 - Clinical course of poliomyelitis and timing of sample collection

#### **Available methods**

- Direct techniques: culture and RT-PCR

#### Samples to collect and sample collection and storage conditions

Sample type

Stool should be collected in a sterile stool container.

Table 2.10 - Samples to collect and collection and shipping conditions – Poliomyelitis

Sample type	Amount	Storage time and conditions	Time frame for shipment to the reference lab	Transport temperature
Stool: 2 samples collected 48 hours apart, within two weeks of symptom onset (intermittent shedding)	Size of a walnut or of the distal phalanx of an adult thumb	Store at +2 °C to +8 °C within a few hours of collection	1 week between collection and arrival at the lab	+2 °C to +8 °C

#### Sample collection procedure

- Hygiene: see Chapter 4.

- **SOP:** see Chapter 3.

Collecting a stool sample, see Section 3.6.1.

#### Test request form and logging

See the poliomyelitis test request form, Chapter 5, Section 5.5. This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected poliomyelitis infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

#### 2.6 PoxVirus

#### The disease

There are several poxviruses that affect humans, including variola (smallpox), the monkeypox virus, and the cowpox virus; all are orthopoxviruses.

**Smallpox** presented in two forms: *Variola major* and *Variola minor*, or alastrim. The WHO declared smallpox eradicated in 1980.

**Monkeypox** resembles smallpox but has milder symptoms. After an episode of fever, headache, and malaise, the patient develops a rash that progresses from macules to papules to vesicles to pustules and then to scabs in about two weeks. All of the lesions in a given area of the body (face, palms, soles of the feet, and sometimes mucous membranes) are the same age. The case fatality rate is no more than 10%, and most deaths are in children 15. Transmission occurs via contact with the blood, body fluids, skin, or mucosal lesions of infected animals (monkeys and rodents), or very close contact with infectious secretions from a patient's respiratory tract or skin lesions, or even from objects recently contaminated by fluids from a patient's lesion. The disease occurs mainly in rural, forested areas.

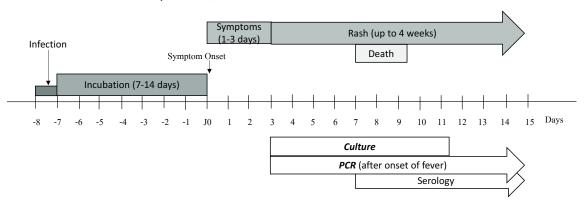


Figure 2.14 - Clinical course of monkeypox and timing of sample collection

**Cowpox** is spread by skin-to-skin contact between animals and humans. It presents as painful erythematous macules with oedema on the face, neck, and hands that progress to become ulcerated, and then necrotic, lesions.

#### **Available methods**

- Direct techniques: **culture and PCR**. Use these if possible.
- Indirect technique: ELISA (IgG and IgM). Cross reactions are possible, which can make results interpretation difficult.

#### Samples to collect and sample collection conditions

If monkeypox virus infection is suspected, contact the reference laboratory, which will issue a **shipment authorisation** and specify the type of sample they want and whether viral transport medium is needed.

#### Samples to collect and sample collection and storage conditions

The type of sample to collect will depend on the stage of the disease.

Skin samples collected at the time of the rash are preferred, as they contain a lot of viral particles.

**Table 2.11** - Samples to collect and collection and shipping conditions — Poxvirus

Test type	Sample type and amount			Maximum time between collection and arrival at the lab	Storage and shipping temperature
Swabs of vesicle or pustule fluid Sample		When vesicles	Immediately transfer the swab	1 week	Ideal: +2 °C to +8 °C
	2 lesions using 1 swab per lesion.	present	pustules are into the transport medium (if applicable)  3 days		+15 °C to +25 °C and protected from light
Culture and PCR  Scabs and membranes covering the vesicle Sample 4 scabs: 2 scabs from 2 different areas of the body	membranes covering the vesicle	When scabs are	Each scab in a	1 week	Ideal: +2 °C to +8 °C
	2 scabs from 2 different areas of	present	sterile cryotube	3 days	+15 °C to +25 °C and protected from light
PCR	Serum: 1 mL	After onset of fever NA		1 week	+2 °C to +8 °C
Serology	Serum: 1 mL	Starting 4 days after onset of rash	NA	1 week	+2 °C to +8 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

To avoid cross contamination, each specimen (scab or membrane) should be stored in its own tube.

#### Transport medium

Some reference laboratories will require that swabs be transported in viral transport medium. Contact the reference lab before collecting the samples to see whether transport medium is needed.

#### Sample collection procedure

- Hygiene: see Chapter 4.

Staff who have contact with suspected monkeypox patients should wear personal protective equipment including:

- Gloves
- A gown and an overgown or disposable coverall
- A respirator (FFP2/N95)
- A face shield or goggles

- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum, see Section 3.3.
  - Collecting scab samples, see Section 3.8.3.
  - Swabbing fluid from a lesion, see Section 3.8.2.

#### Test request form and logging

See the poxvirus test request form, Chapter 5, Section 5.6. This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers

#### **Transport**

Samples for diagnosis or lab monitoring of monkeypox belong to Category A. They must be transported as an infectious substance and labelled UN2814 (see Chapter 6, Section 6.4 for more details).

## 2.7 Measles and rubella

#### The disease

**Measles** is an acute, highly contagious viral infection caused by a virus from the *Paramyxoviridae* family. It is transmitted via the airways.

The disease presents in two phases: a prodromal or catarrhal phase (2 to 4 days) followed by an eruptive phase (4 to 6 days). There are variable complications (neurological, ocular, gastrointestinal, and respiratory) in 70 to 80% of measles cases <sup>16,17</sup>. Deaths are due to those complications.

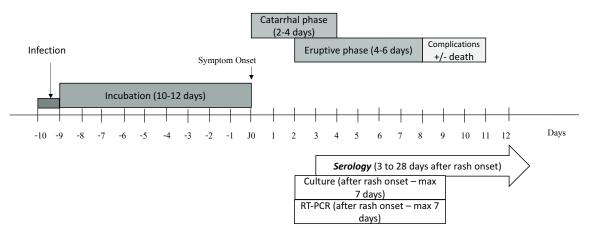


Figure 2.15 - Clinical course of measles and timing of sample collection

**Rubella** is an acute contagious viral infection caused by a virus from the *Togaviridae* family. It is transmitted via airway secretions. The symptoms are similar to those caused by measles, with fever and rash, but less severe and with few complications. This infection is particularly dangerous during the first trimester of pregnancy, as it can lead to miscarriage, foetal death, and congenital malformations <sup>18</sup>.

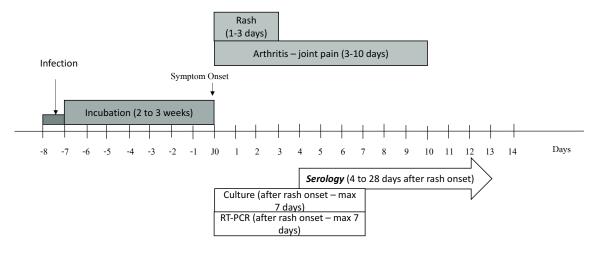


Figure 2.16 - Clinical course of rubella and timing of sample collection

Laboratory confirmation is obligatory for declaring an outbreak. Once an outbreak has been declared in a given geographic area, there is no need for lab confirmation of every case – the clinical picture suffices. If measles testing is negative, always test for rubella.

See Management of a Measles Epidemic.

#### **Available methods**

- Indirect technique: serology: presence of IgM (ELISA). This is the gold standard for outbreak investigation.
- Direct techniques (RT-PCR, sequencing, and culture) are not diagnostic tools, but are used to study the genome or isolate the virus. The samples for those analyses are not covered in this guide.

#### Samples to collect and sample collection and storage conditions

Table 2.12 - Samples to collect and collection and shipping conditions - Measles and rubella

Sample type for ELISA	Amount to collect	Timing of sample collection	Storage conditions prior to shipping	Allowable time before arrival at the lab	Shipping conditions
Serum or plasma (depending on the technique used by the reference lab)	Collect a 4-mL tube of blood for adults and 2 or 4 mL of blood for children	Between day 3 and day 28 after rash onset	+2 °C to +8 °C within an hour of separating the serum/ plasma	Less than 3 days (acceptable up to 7 days)	+2 °C to +8 °C
DBS (capillary	At least 3 well-	Between day 3	Allow to dry for 3 to 4 hours, then ideally store at + 2 °C à +8 °C in a Ziploc bag with silica gel	Good for more than 7 days	+15 °C to +25 °C
blood)*	filled spots	Between day 3 and day 28 after rash onset  Allow to dry for 3 to 4 hours, then can be stored at up to		Maximum 7 days	+15 °C to +25 °C

- Serum: use a 4-mL dry/red Vacutainer® tube and transfer at least 1.5 mL of serum into a cryotube (if more serum is needed, collect several tubes).
- Plasma: use a 4-mL EDTA/purple Vacutainer® tube and transfer at least 1.5 mL of plasma into a cryotube (if more plasma is needed, collect several tubes).
- Avoid haemolysis (a criterion for sample rejection).

<sup>\*</sup> Serological testing that use DBS samples are not available everywhere. Check with the lab in advance.

#### Sample collection procedure

- Hygiene: see Chapter 4.
  - Measles is transmitted via the airways. Aerosols suspended in the air remain infectious for 30 minutes. Unvaccinated people should use a respirator (FFP2/N95)..
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum and plasma, see Section 3.3 and Section 3.4.
  - Collecting dried blood spot (DBS) samples, see Section 3.2.4.

#### Test request form and logging

See the measles and rubella test request form (Chapter 5, Section 5.7). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected measles or rubella infection are Category B samples and are labelled UN3373 (see Chapiter 6, Section 6.3 for more details).

## 2.8 Cholera

#### The disease

Cholera is an acute, highly contagious and potentially fatal intestinal infection caused by *Vibrio cholerae* serogroups O1 and O139. It presents in the form of sudden-onset copious watery diarrhoea that can rapidly lead to severe dehydration and death without quick, appropriate management.

See Management of a Cholera Epidemic.

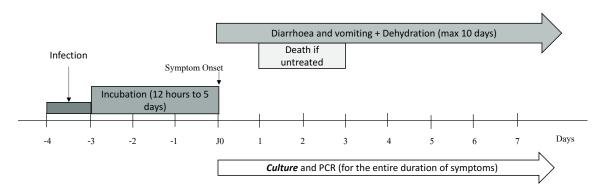


Figure 2.17 - Clinical course of cholera and timing of sample collection

#### Available methods (for outbreak investigation only)

- In the field, a qualitative RDT (antigen test) can be done on a stool sample. It is a test to be used for outbreak investigation only and cannot be used to diagnose individual cases. There are currently no WHO-prequalified rapid cholera tests and no standard tests available in the MSF catalogue. It is possible, however, to order the test as a non-standard item, but only after validation by the MSF operational centre medical director. If necessary, contact the laboratory advisor.
- Direct techniques: culture and drug susceptibility testing, PCR, and antigen test.
- Determination of serogroup by agglutination and serotyping for O1.

#### Samples to collect and conditions for sample collection and storage

#### Sample type

Collect chlorine-free stool directly from the patient (do not use stool from buckets, which are generally chlorinated).

#### - Timing of sample collection

Samples should be collected while the patient has diarrhoea, ideally less than 4 days after symptom onset (to ensure a high bacterial load) and before any antibiotic treatmente.

**Duration and** Allowable condition time between before Sample **Transport** Storage prior collection Shipping **Amount** inoculation medium to shipment and arrival temperature type in the at reference transport **laboratory** medium Ideal: 24 1 wellhours, and no saturated Cary-Blair®\* more than 1 At most < 30 °C\*\* week for Caryswab Chlorine-5 hours at +15 °C protected Blair® free stool 15-20 °C, not to +25 °C\*\* No more than from sunlight dried out 1 well-2 or 3 drops 2 weeks for saturated of normal filter paper in filter paper saline normal saline.

Table 2.13 - Samples to collect and collection and shipping conditions - Cholera

#### Sample collection procedure

- Hygiene: see Chapter 4.
   If using filter paper to collect samples, it is important to disinfect the forceps between each sample and when done collecting the samples.
- **SOP**: see Chapter 3.
  - Collecting stool samples on filter paper, see Section 3.6.3.
    - Check in advance about whether filter paper can used for samples sent to national or regional laboratories, because this sample collection technique is not used everywhere.
  - Faecal swab in transport medium, see Section 3.6.2.
  - Rectal swab in transport medium, see Section 3.6.4.

#### Test request form and logging

See the cholera test request form (Chapter 5, Section 5.8). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected Vibrio cholerae infection are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

<sup>\*</sup> Before use, Cary-Blair® medium should be stored at +5°C to +25°C.

<sup>\*\*</sup> Vibrio are very robust in liquid media. They do not tolerate low temperatures (do not put samples in the cold chain).

## 2.9 Typhoid fever

#### The disease

Typhoid fever is an infection caused by the bacterium *Salmonella typhi*. It is found mainly in overpopulated areas where there is little access to hygiene. The bacteria multiply in the body before entering the bloodstream. The disease is transmitted via the faecal-oral route. The incubation period is 5 to 21 days. The symptoms are variable, with fever and intestinal problems. The disease lasts several weeks and can cause intestinal perforation, septicaemia, and – in the most severe cases – death.

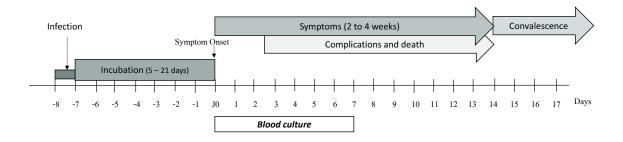


Figure 2.18 - Clinical course of typhoid fever and timing of sample collection

#### **Available methods**

- Direct techniques: culture and drug-susceptibility testing; blood culture for diagnosis.
- Indirect technique: the Widal test (detection of O and H agglutinins) should never be used for diagnostic confirmation due to its low sensitivity and specificity. MSF does not recommend its use.

#### Samples to collect and sample collection conditions

## Sample type Blood sample

#### - Amount to collect

Blood culture: fill the collection bottle as instructed in the table below. The amount must be enough to make up for the low bacterial load in acute typhoid fevers.

Age group	Volume of blood per bottle
Adults and youth ≥ 15 years	10 mL
Children ≥ 2 years	2.5-5 mL
Infant < 2 years	1-2 mL
Newborn (up to 1 month)	0.5-1 mL

#### - Timing of sample collection and sample storage and shippings

Table 2.14 - Samples to collect and collection and shipping conditions - Typhoid fever

Timing of sample collection	Transport/culture medium	Storage time and conditions prior to inoculation	Storage temperature after inoculation (varies depending on the blood culture bottle)*	Allowable time for shipping to the reference lab	Transport time and conditions
As soon as possible after symptom onset and before antibiotic treatment; ideally in the	No transport medium. Inoculate directly into the culture	Collect in the culture medium or transfer immediately	Manual technique (Liofilchem® culture bottle) +36 °C ± 1 °C	4 hours	+36°C±1°C
first 7 days of the illness, because that is when the bacterial load is highest.	medium.	into the culture medium bottle.	Automated technique (BACT/ALERT®): +25 °C		+25 °C



<sup>\*</sup> Check with the lab in advance to find out exactly which blood culture bottles to use.

#### Sample collection procedure

- Hygiene: see Chapter 4.
   Follow the specific procedures for sterile blood culture collection.
- SOP: see Chapter 3.
  - Collecting a blood culture sample, see Section 3.2.3.

#### Test request form and logging

See the typhoid fever test request form (Chapter 5, Section 5.9). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Because the time between sample collection and receipt at the laboratory must be very short, air transport is probably not an option. For your information, samples for suspected typhoid fever, paratyphoid, and salmonellosis are Category B samples and are labelled UN3373, see Chapter 6, Section 6.3 for more details).

## 2.10 Shigellosis

#### The disease

Shigellosis is caused by bacteria from the genus Shigella (S. flexneri, S. boydii, S. sonnei and S. dysenteriae). These bacteria cause dysentery with or without fever, and often severe abdominal and rectal pain. The species that are most common in developing countries and cause the most severe symptoms are S. flexneri and S. dysenteriae serotype 1. Shigella dysenteriae type 1 (Sd1) is the only strain capable of causing large-scale outbreaks, and has the highest case fatality rate (up to 10%)<sup>19</sup>.

Because these species are becoming increasingly resistant to antibiotics, drug-susceptibility testing is essential.

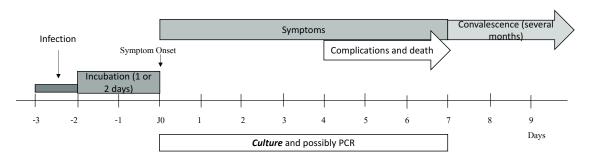


Figure 2.19 - Clinical course of shigellosis and timing of sample collection

#### **Available methods**

Direct technique: culture followed by serogrouping/serotyping to identify Shigella dysenteriae type 1 by agglutination. If the identification result is ambiguous, PCR can be done.

#### Samples to collect and sample collection and storage conditions

Table 2.15 - Échantillons à prélever et conditions du prélèvement et d'envoi – Shigellose

Sample type	Timing of sample collection	Transport medium	Sample storage time before inoculation in transport medium	Sample storage conditions after inoculation	Allowable time for shipping to reference lab	Transport conditions
Faecal or possibly rectal swab	If diarrhoea with mucus and blood, before antibiotic treatment	Amies w/ charcoal (1st choice - ideal for isolating Shigella) Cary-Blair® (2nd choice)*	< 2 hours (very fragile bacteria)	+2 °C to + 8°C	Ideal: 3 days	+2 °C to +8 °C

<sup>\*</sup> Before use, Amies w/charcoal and Cary-Blair® media should be stored at +5 to +25°C.

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Faecal swab in transport medium, see Section 3.6.2.
  - Rectal swab in transport medium, see Section 3.6.4.

#### Test request form and logging

See the shigellosis test request form (Chapter 5, Section 5.10). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected dysentery are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

## 2.11 Pertussis (whooping cough)

#### The disease

Pertussis is a highly contagious respiratory disease caused by *Bordetella pertussis* and *parapertussis bacteria*. It can be fatal in infants and lead to serious complications in young children (pneumonia or neurological disorders).

After the incubation period, pertussis has three phases: a catarrhal phase, a paroxysmal phase characterised by a persistent cough, and a convalescent phase.

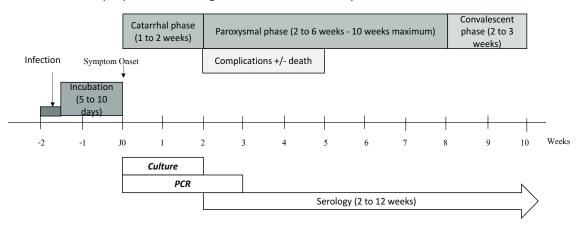


Figure 2.20 - Clinical course of pertussis and timing of sample collection

#### **Available methods**

- Direct techniques: RT-PCR and culture
- Indirect technique: while it is possible to do a serological test-ELISA (targets the antibodies against *Bordetella pertussis* toxin), it has no diagnostic value if the patient has been vaccinated, since there is no difference between the vaccine-induced antibodies and antibodies due to the disease

# Samples to collect according to laboratory method and the type of sample, storage, and shipping

<b>Table 2.16</b> - Sa	mples to co	llect and co	llection and	l shipping	conditions – F	Pertussis

Test	Sample type	Amount – Transport medium	Timing of sample collection	Storage temperature	Shipment time to lab	Shipping temperature
Culture	Nasal or nasopharyngeal aspirate (infants/ children) Sputum	At least 500 microliters in a sterile specimen container	Within 2 weeks of symptom onset (to be strictly adhered to)	+15 °C to +25 °C	Ideal: within 48 hrs. Maximum: 5 days	+15 °C to +25 °C
	Naso- pharyngeal swab	1 swab in Amies w/ Charcoal*	,			

Test	Sample type	Amount – Transport medium	Timing of sample collection	Storage temperature	Shipment time to lab	Shipping temperature
PCR	Nasal or naso- pharyngeal aspirate (infants/ children)	At least 500 μL in a sterile specimen container	Within 2 weeks of symptom onset	+2 °C to +8 °C As soon as	Ideal: within 48 hrs.	+2 °C to +8 °C
	Sputum		(to be strictly adhered to)	possible after collection	Max: 5 days	
	Naso- pharyngeal swab	1 swab in Amies w/ Charcoal*	,			

<sup>\*</sup> Before use, Amies w/Charcoal should be stored at +5°C to +25°C.

Bordetella pertussis and parapertussis are fragile bacteria and should be transferred into the transport medium (if applicable) at a low temperature as quickly as possible.

#### Sample collection procedure

- Hygiene: see Chapter 4.
   The disease is spread via droplets. A surgical mask is required.
- SOP: see Chapter 3.
  - Collecting nasopharyngeal aspirate, see Section 3.5.1.
  - Collecting a nasopharyngeal swab, see Section 3.5.2.
  - Collecting a sputum sample, see Section 3.5.6.

#### Test request form and logging

See the pertussis test request form, (Chapter 5, Section 5.11). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected pertussis are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

## 2.12 Diphtheria



Diphtheria is highly contagious. It is imperative to wear a surgical mask and gloves when handling samples or when in contact with patients.

#### **Pathologie**

Diphtheria is a highly contagious infection due to a corynebacterium from the *diphtheriae* species complex (*Corynebacterium diphtheriae*, *Corynebacterium ulcerans*, or *Corynebacterium pseudotuberculosis*) and to dissemination of diphtheria toxin in the body.

The respiratory form is characterised by whitish "pseudomembranes" on the tonsils that can interfere with breathing. The toxin can also cause cardiac, neurological, and renal complications. There is a cutaneous form as well.

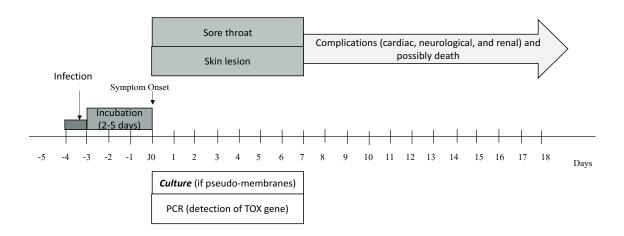


Figure 2.21 - Clinical course of diphtheria and timing of sample collection

#### **Available methods**

 Direct techniques: culture and drug-susceptibility testing, and PCR detection of diphtheria toxin on cultures.

#### Samples to collect and sample collection and storage conditions

**Table 2.17** - Samples to collect and collection and shipping conditions – Diphtheria

Disease type	Sample type	Sample collection timetable	Transport medium	Time between sample collection and transfer into transport medium	Storage temperature	Time between sample collection and arrival at reference laboratory	Shipping temperature
Respiratory diphtheria*	Throat swab**	As soon as symptoms appear	Amies w/ charcoal or Cary- Blair®***	Immediately	+15 °C to +25 °C****	Within 48 hrs.	+15 °C to +25 °C****
	Nasopharyngeal swab						
Cutaneous diphtheria	Lesion swab	As soon as symptoms appear	Amies w/ charcoal or Cary- Blair®***	Immediately	+15 °C to +25 °C****	Within 48 hrs.	+15 °C to +25 °C****

<sup>\*</sup> Collecting samples from different sites can improve the chances for laboratory confirmation.

#### Sample collection procedure

- Hygiene: see Chapter 4.

Transmission of respiratory diphtheria is via droplets and a surgical mask is required. Gloves are also required.

- SOP: see Chapter 3.
  - Collecting a throat swab, see Section 3.5.4.
  - Collecting a nasopharyngeal swab, see Section 3.5.2.
  - Collecting a skin and lesion swab, see Section 3.8.1.

#### Test request form and logging

See the diphtheria test request form (Chapter 5, Section 5.12). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected diphtheria are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

<sup>\*\*</sup> Swab the pseudomembranes, if present.

<sup>\*\*\*</sup> Prior to use, Amies w/charcoal and Cary-Blair® media should be stored at +5 to +25°C.

<sup>\*\*\*\*</sup> Diphtheria bacteria are robust and can tolerate temperatures up to 25°C.

## 2.13 Leptospirosis

#### The disease

Leptospirosis is a zoonosis caused by spirochetes from the genus *Leptospira*; it affects many domestic and wild animals, especially rodents (brown rats).

Human infection is generally indirect, via contact between rat urine-contaminated water and broken skin. The clinical picture is varied; but there is a moderate form (most common) and a severe form (jaundice and multiple organ involvement). The disease usually starts with a sudden-onset fever, muscle pain, and headache. The severe form occurs in 5 to 10% of cases with a case fatality rate of 15 to 20%, and up to 50% in patients with pulmonary complication<sup>20,21</sup>.

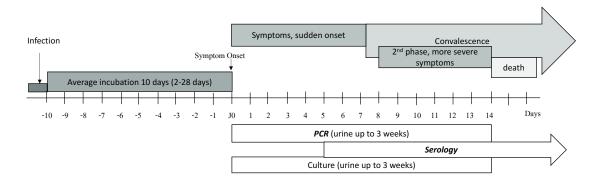


Figure 2.22 - Clinical course of leptospirosis and timing of sample collection

#### **Available methods**

- Direct technique: PCR
- Cultures are also possible. However, because it takes three months to get the results, culture
  is not used for diagnosis when an outbreak is suspected.
- Indirect technique: Serology using ELISA and microscopic agglutination test (MAT

#### Samples to collect and collection and storage conditions

Table 2.18 - Samples to collect and collection and shipping conditions - Leptospirosis

	Sample type	Timing of sample collection	Storage temperature	Time between collection and arrival at the lab	Shipping temperature
Serology	Serum: 0.5 mL	Starting Day 5 after symptom onset. Repeat 1 to 3 weeks later, depending on the reference lab's recommendations.	+2 °C to +8 °C	No specific time (ideal: less than one week)	+2 °C to +8 °C

	Sample type	Timing of sample collection	Storage temperature	Time between collection and arrival at the lab	Shipping temperature
PCR	Urine: 1 mL Within 3 weeks of symptom onset.  Before antibiotic therapy.		+2 °C to +8 °C	No specific time	+2 °C to +8 °C
	At least 0.5 mL of whole blood with an EDTA tube	Within 2 weeks of symptom onset.	+2 °C to +8 °C	No specific timer	+2 °C to +8 °C
	CSF: 0.2 mL	Before antibiotic therapy			

- Serum: use a 4-mL dry/red Vacutainer<sup>®</sup> tube and transfer at least 0.5 mL of serum into a cryotube.
- Avoid haemolysis (a criterion for sample rejection).

#### Sample collection procedure

- Hygiene: see Chapter 4.
- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Preparing serum, see Section 3.3.
  - Collecting a urine sample, see Section 3.7.
  - Collecting a CSF sample, see Section 3.10.1 and transferring CSF into a cryotube, see Section 3.10.3.

#### Test request form and logging

See the leptospirosis test request form (Chapter 5, Section 5.13). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected leptospirosis are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

## 2.14 Meningitis

#### The disease

Bacterial meningitis is an acute infection of the meninges that can potentially cause brain damage and irreversible neurological and auditory sequelae. It is transmitted via saliva droplets.

The most common causative agent varies with age and/or context. In an epidemic context, in the Sahel, it is generally meningococcus (Neisseria meningitidis), which affects every age group but primarily people under 30 years of age.

At other times, it can involve any of the pathogens typically responsible for meningitis, particularly among young children.

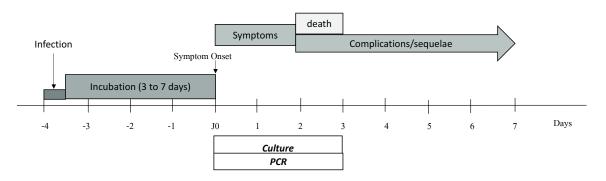


Figure 2.23 - Clinical course of meningitis and timing of sample collection

N. meningitidis serogrouping is essential at the start of an outbreak for choosing an appropriate vaccine for the response.

In an epidemic context, once the meningococcal aetiology has been confirmed, lumbar puncture (LP) is not done routinely for new cases. It is useful for complete epidemiological surveillance to document the situation (after the introduction of a new vaccine, the emergence of or an unusual increase in a microorganism, etc.). The surveillance protocol should be discussed on a case-by-case basis with the medical department and the epidemiologists.



See Management of epidemic meningococcal meningitis

#### Available methods

 In the field, a Pastorex<sup>®</sup>-type latex agglutination test can be used to detect specific bacterial antigens (store the sample in the cold chain for no more than 48 hours). The test is used to confirm an outbreak, and not for diagnosing individuals or declaring an epidemic. Additional tests can also be performed on CSF (Gram stain, Pandy test, blood cell count, and glucose levels.

See MSF Laboratory Manual.

- Direct techniques: microscopy (Gram stain), antigen test, **PCR**, culture, drug-susceptibility testing, and clone typing/identification.

#### Samples to collect and conditions for sample collection and storage

Table 2.19 - Samples to collect and collection and shipping conditions – Meningitis

Test type	Sample type and amount	Timing of sample collection	Transport medium	Sample storage conditions before inoculation in transport medium	Sample storage conditions after inoculation in transport medium	Maximum time before arrival at the laboratory	Transport conditions
Culture	CSF: 0.5 – 1 mL (10 to 20 drops)	Febrile disease, before antibiotic therapy*	Trans- Isolate**	Maximum 1 hour at +15 °C to +25 °C	< 40 °C protected from direct sunlight. DO NOT REFRIGERATE	3 weeks with ventilation	<40 °C 6 to 7 days without ventilation
PCR and identification of clones	CSF: 0.2 mL minimum (4 drops minimum)	Febrile disease, before antibiotic therapy*	NA	NA	+2 °C to +8 °C	3 weeks	+2 °C to +8 °C

<sup>\*</sup> The Pastorex® test can be done after an initial dose of antibiotics, but the test will be less sensitive.

The use of TI transport medium for CSF [STSSTRTUTI1] is validated and supplied only for confirmation of a meningitis outbreak in the meningitis belt (for identifying strains) and for epidemic surveillance, but not for individual clinical diagnosis tests (CSF culture) and only for MSF projects, with the Norwegian Institute of Public Health laboratory in Oslo (WHO Collaborating Centre for Reference and Research on Meningococci). In non-meningitis belt countries, TI medium must be ordered through the WHO or the Ministry of Health



See the Laboratory Working Group communication paper - December 2018.

#### Sample collection procedure

- Hygiene: see Chapter 4.
   Respect the principles of sterility and aseptic technique when Collecting a CSF sample samples.
- SOP: see Chapter 3.
  - Inoculating Trans-Isolate medium with CSF, see Section 3.10.2.
  - Transferring CSF into a cryotube, see Section 3.10.3.

<sup>\*\*</sup> Trans-Isolate (TI) medium: requires cold chain storage (+2°C to +8°C) prior to use. It must be at room temperature before inoculation, because the sample must be stored at room temperature (meningococci are very sensitive to the cold). The normal appearance of TI is that of a light yellow, translucent fluid; do not use if there is colony growth on the black agar, or if the fluid is cloudy.

#### Test request form and logging

See the meningitis test request form, (Chapter 5, Section 5.14). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers (see "Management of epidemic meningococcal meningitis" – Appendix 1).

#### **Transport**

Samples for suspected meningitis are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3).

## 2.15 Plague

#### The disease

Plague is a zoonosis caused by a bacterium (Yersinia pestis) that mainly affects rodents. Transmission to humans can be via flea bites or rodent contact with broken skin.

There are three main clinical forms: bubonic plague, septicaemic plague, and pneumonic plague, which is highly contagious. Human-to-human transmission occurs via flea bites and via the airways (for the pneumonic form).

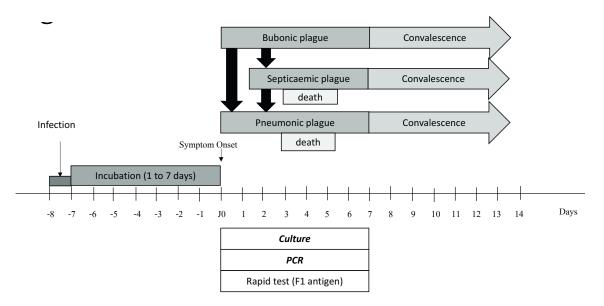


Figure 2.24 - Clinical course of plague and timing of sample collection



Pneumonic plague is highly contagious. Wearing a respirator (FFP2/N95 masks) and gloves is imperative when handling samples or having any contact with patients.

#### Available methods

- Direct techniques: Rapid diagnostic test (F1 antigen detection), culture, and PCR.

Reference laboratories use RDTs that can detect Y. pestis-specific F1 antigen using various types of samples (bubo fluid and sputum). Those RDTs are effective for bubonic plague, but less so for pneumonic plague (due to cross reactions in sputum).

#### Samples to collect and sample collection and storage conditions

**Table 2.20** - Samples to collect and collection and shipping condition – Plague

Disease type	Sample type and amount	Transport medium**	Timing of sample collection	Storage temperature and conditions***	Time between collection and arrival at reference laboratory	Shipping temperature ***
Bubonic plague	Bubo aspirate or oozing:	Cary-Blair®	When the patient is	+2 °C to +8 °C  For swab in normal saline:	3 days	+2 °C to +8 °C
	1 bubo swab	Sterile normal saline	symptomatic	DO NOT LET SAMPLE DRY OUT		
	Deep sputum: 2 mL	NA		+2 °C to +8 °C	or swab in rmal saline: 3 days O NOT LET	+2 °C to +8 °C
Pneumonic plague*	1 sputum swab	Cary Blair®	When the patient is symptomatic	For swab in normal saline: DO NOT LET		
		Sterile normal saline		SAMPLE DRY OUT		
Septicaemic plague	Whole blood: 4 mL	NA	When the patient is symptomatic	+2 °C to +8 °C  For swab in normal saline: DO NOT LET SAMPLE DRY OUT	3 days	+2 °C to +8 °C

Contact headquarters medical advisors if considering.

If Cary-Blair® medium is not available and the samples are collected on swabs, the latter should be moistened with sterile water or sterile normal saline. Add enough liquid to ensure that the swabs are soaking in liquid, so that they do not dry out.

#### Sample collection procedure

- Hygiene: see Chapter 4.

Since the plague is highly contagious, wear the following when collecting a sample: a respirator (FFP2/N95 masks), gloves, goggles, and sturdy, closed shoes. Handle waste carefully; use a 0.5% chlorine solution for disinfection.

- SOP: see Chapter 3.
  - Collecting a venous blood sample with a Vacutainer® system, see Section 3.2.1.
  - Aspirating a plague bubo, see Section 3.9.1.
  - Collecting pus from an oozing plague bubo, see Section 3.9.2.
  - Collecting a sputum sample and swabbing for pneumonic plague, see Section 3.5.7.

<sup>\*\*</sup> Cary-Blair® is the recommended transport medium for swabs of bubo fluid (bubonic plague) or sputum (pneumonic plague). Cary-Blair® medium should be stored at +5°C to +25°C prior to use.

<sup>\*\*\*</sup> Samples for plague diagnosis should be stored at +2°C to +8°C, whether stored with or without Cary-Blair® transport medium. The plague bacillus is stable and multiplies at that temperature.

#### Fiche de demande d'examen et enregistrement

See the plague test request form (Chapter 5, Section 5.15). This form must accompany the sample during shipment if the reference laboratory's information form is not available for the shipment.

Sample information must be entered in the registers.

#### **Transport**

Samples for suspected plague are Category B samples and are labelled UN3373 (see Chapter 6, Section 6.3 for more details).

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# 3.1 Introduction

All samples should be collected by trained personnel in an environment where there is no risk of spreading infection.

Hand hygiene is an essential part of sample collection, both to protect the collector and to avoid contaminating the samples. All the hand hygiene procedures described below are an integral part of the sample collection protocol. However, it is essential that they be incorporated into the more general organisation of care.

The use of personal protective equipment (PPE) should be considered whenever a sample is collected, according to the infectious potential of the agent being investigated, the risk of spraying or splashing, and the type of sample see Chapter 4, Section 4.2.

# 3.2 Venous blood

# 3.2.1 Collecting blood samples using a Vacutainer® system (non-haemorrhagic fever context)

# **Equipment for outpatient sample collection**

- ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W], 1<sup>st</sup> choice (for other choices, see chapter 4, Section 4.5.2)
- CHLORHEXIDINE digluconate 2%, aqueous solution, 100 mL, bot. [DEXTCHLH2AS], for neonatology
- MARKER, permanent, black, fine point [ELABMARK1B-]
- (tube Ø 13/15 mm, 5 mL) RACK [ELABTUBE12R]
- TOURNIQUET, elastic, 100 x 1.8 cm [EMEQTOUR1--]
- TRAY, DRESSING, 30 x 20 x 3 cm, stainless steel [EMEQTRAD3--]
- SUCROSE, 24% oral solution, 2 mL, vial. [SDDCSUCR2V2], for children < 6 months
- COMPRESS, NON WOVEN, 4 plies, 7.5 cm, non sterile [SDRECOMN7N-]
- COTTON WOOL, hydrophilic, roll, 500 g [SDRECOTW5R-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- CONTAINER, needles/syringes [SINSCONT+++]
- GLOVE, EXAMINATION, latex, s.u. non sterile [SMSUGLOE1--]
- (blds.syst.) TUBE, VACUUM, plastic, K2EDTA, 2 mL, purple [STSSBSVT2E-]
- (blds.syst.) TUBE, VACUUM, plastic, K2EDTA, 4 mL, purple [STSSBSVT5E-]
- (blds.syst.) TUBE, VACUUM, plastic, Li-HEPARIN, 2 mL green [STSSBSVT2HL]
- (blds.syst.) TUBE, VACUUM, plastic, Li-HEPARIN, 4 mL green [STSSBSVT5HL]
- (blds.syst.) TUBE, VACUUM, plastic, SERUM, 2 mL, red [STSSBSVT2S-]
- (blds.syst.) TUBE, VACUUM, plastic, SERUM, 4 mL, red [STSSBSVT4S-]
- (blds. syst.) HOLDER for VACUUM TUBE with needle ejector [STSSBSVVH1-]
- (blds.syst.) NEEDLE, sterile, 21G (Vacutainer) [STSSBSVVN21]
- (blds.syst.) SAMPLING SET, with wings, 23G (Vacutainer) [STSSBSVVN23W]

#### Notes:

- This list refers mainly to venous blood collected at the lab or in an outpatient setting, not in an inpatient setting.
- The equipment for sample collection using syringes and needles has been removed from the list. Vacutainer® collection is preferred for safety reasons.

See the SOP in the Nursing Care Procedures manual for more details on inpatient or syringe/needle sample collection.

#### Before collecting the sample

- Position the patient comfortably with his arm angled downward and supported by an armrest.
- Confirm the patient's identity and make sure that it matches the doctor's blood draw order.
- Explain the procedure to the patient and confirm his medical history, allergies, and history
  of previous sample collection; give the patient an opportunity to ask questions and obtain
  his verbal consent (which should be documented in the chart).

- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Perform hand hygiene.
- Assemble the sample collection equipment on a tray or disinfected treatment trolley.
- Label the tubes with the patient's unique identification number and the collection date and time.
- Locate the vein: while this step is not essential, it may turn out to be necessary if the patient's veins are difficult to see.
- Perform hand hygiene.
- Apply the tourniquet to the chosen limb (four finger widths above the venepuncture site for adults and two finger widths above for newborns and paediatric patients).
- Choose the venepuncture site by palpation. Ask the patient to clench/unclench his fist. Do
  not leave the tourniquet on for too long to avoid venous stasis, which can result in increased
  levels of substances such as haemoglobin, plasma proteins, and potassium in the blood.
  Once the vein has been identified, remove the tourniquet

### Sample collection procedure

- Perform hand hygiene.
- Connect the collection tube (Vacutainer®) holder to the blood collection needle.
- For infants (under 6 months):
  - Consider giving an oral sucrose solution 2-3 minutes before venepuncture; if the procedure lasts more than 5 minutes, a second dose can be given.
  - Consider immobilising the child with a towel or asking an assistant for help.
- Perform hand hygiene and put on a pair of nonsterile gloves.
- Disinfect the patient's skin with an antiseptic solution-soaked compress, using a back-andforth motion, for 30 seconds. Let dry.
- Apply the tourniquet.
- Uncap the needle and turn it so the bevel faces up. Secure the vein by applying traction with your thumb, taking care not to touch the insertion site.
- Using your dominant hand, in one smooth motion, insert the needle into the vein at an angle of about 15 to 30°.
- Reduce the insertion angle of the needle as soon as you feel it pierce the wall of the vein (or blood flows into the tubing, if using a winged needle).
- Begin collecting the sample by pushing the first vacuum tube into the tube holder until its cap is pierced.
- Fill, according to the vacuum/to the mark indicated, the number of tubes needed; avoid
  moving the needle in the vein when switching the tubes in the holder. Remove the tubes
  once the required amount of blood had been drawn.
- When the last collection tube has been filled, loosen the tourniquet before detaching that final tube.
- Place dry cotton wool on the puncture site and remove the needle. Apply enough pressure
  on the cotton wool to stop the bleeding. If bleeding lasts more than a minute, you can place
  medical tape over the cotton wool. The nurse can also ask the patient to hold the dressing
  until the bleeding stops. Never bend the elbow (this increases the risk of haematoma).
- Immediately place the needle in a sharps collector and properly dispose of the other waste using the usual procedures.
- Gently invert the tubes 5 to 10 times.
- Remove the nonsterile gloves and throw them with the other waste into the appropriate trash bins.
- Perform hand hygiene.

### After collecting the sample

- Place the tubes into the specimen transport bag or box.
- Store the tubes away from direct sunlight. Follow the usual procedure for transporting samples to the laboratory.

# 3.2.2 Collecting a venous blood sample for <u>suspected haemorrhagic fever</u> with a safety- Vacutainer® system

Whenever a haemorrhagic fever is suspected, personal protective equipment is essential, two people are needed for sample collection, and samples must be triple-packaged. **Suspected cases should always be placed in an isolation zone.** 

### **General equipment**

See blood sample collection (see Section 3.2.1).

# **Special equipment**

- BAG, plastic, 14 x 17 cm, re-sealable zipper [SMSUBAGP14-], or transport tube transmitted by the laboratory
- (blds. syst.) SAFETY TUBE HOLDER, retractable, semi-aut,s.u. [STSSBSVSRH2]
- (blds.syst.) SAFETY SAMPLING SET, retractable, 23G [STSSBSVSRS23]
- (blds.syst.) SAFETY SAMPLING SET + HOLDER, retractable, 23G [STSSBSVSRS23H]
- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]

See also KMEDMSAM6-- MODULE, SAFETY BLOOD SAMPLING, 100 samples.

#### Preparation before entering the patient's isolation zone

- Assemble the sample collection equipment (see lists above) on a disinfected tray, along with:
  - A plastic bucket or container with 0.5% chlorine solution for transport
  - A spray bottle with 0.5% chlorine solution (for decontamination or accidental exposure)
- Label all of the collection tubes and lab request forms (with the unique patient identification number, date, and time) before entering the isolation zone.
- Put on personal protective equipment.
- Enter in pairs (a medical person and an assistant). The second person (doctor, nurse, lab technician, or hygienist) helps the collector, passing the equipment and decontaminating with the spray bottle. The assistant stays close to the collector.

#### In the isolation zone

- Confirm the patient's identity and make sure that it matches the doctor's blood draw order.
- Explain the procedure to the patient and confirm his medical history, allergies, and history
  of frequent sample collection. Give the patient an opportunity to ask questions and obtain
  his verbal consent (which should be documented in the chart).
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Place all of the sample collection equipment next to the bed.
- Position the patient correctly with his arm angled downward and supported on an armrest.
   If the patient is agitated, postpone the blood draw to a later time. If the blood draw is essential, consider sedation.

- The collector prepares the venepuncture site, placing a tourniquet on the patient's arm.
- Disinfect.
- Collect the sample.
- The assistant holds out the collection tube(s) to the collector and then takes the collected samples (1<sup>st</sup> packaging). Do not spray the collection tube with 0.5% chlorine, as this can cause contamination when the tube is opened.
- Withdraw the needle and retract it directly into the tube holder. Throw the holder/needle combination into the sharps collector.
- Slide the tube into the 1<sup>st</sup> Ziploc bag (2° packaging). Spray the outside of the Ziploc bag and the collector's hands with 0.5% chlorine. If there are several samples, the Ziploc bags should be placed in a larger bag or in a larger container. Spray the outside of that with 0.5% chlorine solution as well.

### Acheminement du prélèvement hors de la zone d'isolement

- The collector passes his arm over the isolation boundary and places the samples in the shipping box (30 packaging) positioned within his reach, without touching anything.
- Lastly, the outside of the shipping box (which is located outside the isolation zone) should be sprayed with 0.5% chlorine solution before the box is closed up

# 3.2.3 Collecting blood culture samples



Respect infection control precautions to prevent contamination.

- If the project uses an outside laboratory, check the type of vial it uses and modify the equipment accordingly.
- For children with malaria or malnutrition, only one blood culture sample can be collected. It
  is better to put the maximum amount in a single blood culture vial than to split the amount
  of blood into two separate vials.
- For adults, collect at least two blood cultures at a time on two separates sites.
- The amount required will depend on the patient's age, as described in the table below:

Age group	Volume of blood per vial	
Child ≥15 years and adults	10 mL	
Children ≥ 2 years	2.5-5 mL	
Infant < 2 years	1-2 mL	
Newborn (up to 1 month)	0.5-1 mL	

### **Equipment for collecting blood cultures samples (automatic-read vials)**

- ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W]
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, col.sol.,250 mL, bot. [DEXTCHLHA2S2C]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- (tube Ø 13/15 mm, 5 mL) RACK [ELABTUBE12R]

- (MDS BacT/ALERT 3D 60) ADAPTOR Luer for sampling sets [ELAEMDSC201]
- MICROBIAL DECTECTION SYSTEM (BacT/ALERT 3D 60) [ELAEMDSE2--]
- MASK, SURGICAL, IIR type, s.u. [ELINMASS3--]
- TOURNIQUET, elastic, 100 x 1.8 cm [EMEQTOUR1--]
- TRAY, DRESSING, 30 x 20 x 3 cm, stainless steel [EMEQTRAD3--]
- BLOOD CULTURE BacT/ALERT Paed (PF Plus), bot. [BMX-410853] [SBCMRDTUBAPPF]
- COMPRESS, NON WOVEN, 4 plies, 7.5 cm, non sterile [SDRECOMN7N-]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- CONTAINER, needles/syringes, 5 L, cardboard for incineration [SINSCONT5C-]
- BAG, plastic, for health card, 16x22 cm, re-sealable zipper [SMSUBAGP16-]
- GLOVE, EXAMINATION, latex, s.u. non sterile [SMSUGLOE1++]
- (blds.syst.) NEEDLE, sterile, 21G (Vacutainer) [STSSBSVVN21]
- (blds.syst.) SAMPLING SET, with wings, 23G (Vacutainer) [STSSBSVVN23W]

*Note:* the vials for automated blood culture systems available at MSF laboratories are paediatrics specific.

# Collecting blood culture samples with vials for automated systems

This procedure describes the steps for collecting venous blood with a direct device, that is, a blood culture tube holder directly connected to a collection needle or a winged needle.

Precautions need to be taken to prevent sample contamination by skin flora (see procedure below).

#### Before collecting the sample

- Confirm the patient's identity and make sure that it matches the doctor's blood draw order.
- Explain the procedure to the patient and confirm his medical history, allergies, and history
  of frequent sample collection. Give the patient an opportunity to ask questions and obtain
  his verbal consent.
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Assemble the sample collection equipment on a tray or disinfected treatment trolley (see above).
- Label the tubes with the patient's unique identification number, the collection date and time, and the volume of blood.
- Locate the vein.
- Perform hand hygiene.
- Apply the tourniquet to the chosen limb (four finger widths above the venepuncture site for adults and two finger widths above for newborns and paediatric patients).
- Choose the venepuncture site by palpation. Ask the patient to clench/unclench his fist. Do
  not leave the tourniquet on for too long to avoid venous stasis, which can result in increased
  levels of substances such as haemoglobin, plasma proteins, and potassium in the blood.
  Once the vein has been identified, remove the tourniquet.

#### Sample collection procedure

- Using nonsterile compresses, clean a generous area of the skin around the venepuncture site with soap and clean water. Let dry.
- Perform hand hygiene.
- Disinfect the collection tube holder using a compress soaked in chlorhexidine/alcohol solution.
- Connect the collection tube holder to the blood collection needle or the winged needle.

- Prepare a batch of chlorhexidine-soaked sterile compresses.
- Remove the plastic flip-cap from the blood culture vial using a compress soaked in chlorhexidine/alcohol solution; disinfect the top of the blood culture vial by rubbing for 15 seconds and leave the compress on the septum.
- Ask the patient to turn and keep his head turned toward the arm opposite the collection to prevent sample contamination.
- For infants:
  - Consider giving an oral sucrose solution 2-3 minutes before venepuncture. Disinfect the skin with a 2% aqueous chlorhexidine solution.
  - Consider immobilising the child with a towel or asking an assistant for help. Ideally, it's a parent who immobilises the child and keeps his head turned toward the arm opposite the collection.
- Perform hand hygiene and put on a pair of nonsterile gloves.
- Disinfect the patient's skin with a chlorhexidine solution-soaked compress, using a backand-forth motion, for 30 seconds. Let dry.
- Apply the tourniquet.
- Uncap the needle and turn it so the bevel faces up. Secure the vein by applying traction with your thumb, taking care not to touch the insertion site.
- Using your dominant hand, in one smooth motion, insert the needle into the vein at an angle of about 15 to 30°.
- Reduce the insertion angle of the needle as soon as you feel it pierce the wall of the vein (or blood flows into the tubing, if using a winged needle), and then, if possible, push the needle slightly deeper into the vein.
- Remove the compress from the septum and begin drawing the sample by pushing the blood culture vial into the collection tube holder until it pierces the vial's septum.
- Fill the vial with the required amount of blood.
- Once the vial is filled, loosen the tourniquet before detaching the vial from the holder.
- Place a dry compress over the venepuncture site and withdraw the needle. Apply enough
  pressure on the compress to stop the bleeding. If bleeding lasts more than a minute, place
  medical tape over the compress.
- The nurse can also ask the patient to hold the dressing until the bleeding stops. Never bend the elbow (this increases the risk of haematoma).
- Immediately place the needle in a sharps collector and properly dispose of the other waste using the usual procedures.
- Gently mix the contents of the vials to blend the blood and the medium. Do not invert the vials.
- Remove the nonsterile gloves and throw them with the other waste into the appropriate trash bin.
- Perform hand hygiene.
- Note the volume of blood transferred on the blood culture vial.

#### After collecting the sample

- Clean/disinfect and put away all of the equipment used.
- Perform hand hygiene.
- Place the vials into the specimen transport bag or box.
- Never refrigerate a blood culture vial.
- Follow the normal procedure for transporting samples to the laboratory.

# **Equipment for collecting blood culture samples (by syringe)**

- ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W]
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, col.s ol.,2 50 mL, bot. [DEXTCHLHA2S2C]

- MARKER, permanent, black, fine point [ELABMARK1B-]
- (tube Ø 13/15 mm, 5 mL) RACK [ELABTUBE12R]
- MASK, SURGICAL, IIR type, s.u. [ELINMASS3--]
- TOURNIQUET, elastic, 100 x 1.8 cm [EMEQTOUR1--]
- TRAY, DRESSING, 30 x 20 x 3 cm, stainless steel [EMEQTRAD3--]
- BLOOD CULTURE AEROBIC, 6 x 80 mL, bot. [Liofilchem-490010] [SBCMRDTUBAEL]
- BLOOD CULTURE AEROBIC NEONAT., 6x9 mL,bot [Liofilchem-490050] [SBCMRDTUBAELN]
- BLOOD CULTURE AEROBIC PED., 6x40 mL,bot. [Liofilchem-490030] [SBCMRDTUBAELP]
- SUCROSE, 24% oral solution, 2 mL, vial.[SDDCSUCR2V2], for children < 6 months</li>
- COMPRESS, NON WOVEN, 4 plies, 7.5 cm, non sterile [SDRECOMN7N-]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- CONTAINER, needles/syringes, 5 L, cardboard for incineration [SINSCONT5C-]
- NEEDLE, s.u., Luer, 19G (1.1 x 40 mm) cream, IV [SINSNEED19-]
- NEEDLE, s.u., Luer, 23G (0.6 x 30 mm) blue, SC, IM child [SINSNEED23-]
- SYRINGE, s.u., Luer, 5 mL [SINSSYDL05-]
- SYRINGE, s.u., Luer, 10 mL [SINSSYDL10-]
- BAG, plastic, for health card, 16x22 cm, re-sealable zipper [SMSUBAGP16-]
- GLOVE, EXAMINATION, latex, s.u. non sterile [SMSUGLOE1++]

*Note:* The vials for automated blood culture systems available at MSF laboratories are paediatrics specific.

# Collecting blood culture samples with a syringe

This procedure describes the steps for collecting venous blood with a manual device, that is, a syringe connected to a winged or 19G needle and a manual blood culture vial. Blood collected in this manner is subsequently transferred from the syringe into the blood culture vial.

Precautions are needed to prevent sample contamination by skin flora (see procedure below).

#### Before collecting the sample

- Confirm the patient's identity and make sure that it matches the doctor's blood draw order.
- Explain the procedure to the patient and confirm his medical history, allergies, and history
  of frequent sample collection. Give the patient an opportunity to ask questions and obtain
  his verbal consent (which should be documented in the chart).
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Assemble the sample collection equipment on a tray or disinfected treatment trolley (see list above).
- Label the blood culture vial with the patient's unique identification number and the collection date and time.
- Locate the vein.
- Perform hand hygiene.
- Apply the tourniquet to the chosen limb (four finger widths above the venepuncture site for adults and two finger widths above for newborns and paediatric patients).
- Choose the venepuncture site by palpation. Ask the patient to clench/unclench his fist. Do
  not leave the tourniquet on for too long to avoid venous stasis, which can result in increased
  levels of substances such as haemoglobin, plasma proteins, and potassium in the blood.
  Once the vein has been identified, remove the tourniquet.

#### Sample collection procedure

- Using nonsterile compresses, clean a generous area of skin around the venepuncture site with soap and clean water. Let dry.
- Perform hand hygiene.
- Connect the syringe to the 19G or winged needle and put the entire thing back in the packaging.
- Prepare a batch of chlorhexidine-soaked sterile compresses.
- Remove the plastic flip-cap from the blood culture vial using a compress soaked in chlorhexidine/alcohol solution; disinfect the top of the blood culture vial by rubbing for 15 seconds and leave the compress on the septum.
- Ask the patient to turn and keep his head turned toward the arm opposite the collection to prevent contamination of the sample.
- For infants:
  - Consider giving an oral sucrose solution 2-3 minutes before venepuncture. Disinfect the skin with a 2% aqueous chlorhexidine solution.
  - Consider immobilising the child with a towel or asking an assistant for help. Ideally, it's a
    parent who immobilises the child and keeps his head turned toward the arm opposite the
    collection.
- Perform hand hygiene and put on a pair of nonsterile gloves.
- Disinfect the patient's skin with a chlorhexidine solution-soaked compress, using a backand-forth motion, for 30 seconds. Let dry.
- Apply the tourniquet.
- Uncap the needle and turn it so the bevel faces up. Secure the vein by applying traction with your thumb, taking care not to touch the insertion site.
- Using your dominant hand, in one smooth motion, insert the needle into the vein at an angle of about 15 to 30°.
- Reduce the insertion angle of the needle as soon as you feel it pierce the wall of the vein (or blood flows into the tubing, if using a winged needle), and then, if possible, push the needle slightly deeper into the vein.
- Begin collecting the sample by pulling the piston on the syringe.
- Fill the syringe with the required amount of blood.
- Once the required amount of blood is in the syringe, loosen the tourniquet.
- Place a dry compress on the venepuncture site and withdraw the needle. Apply enough
  pressure on the compress to stop the bleeding. If bleeding lasts more than a minute, you
  can place some adhesive tape over the compress. The nurse can also ask the patient to
  hold the dressing until the bleeding stops. Never bend the elbow (this increases the risk of
  haematoma).
- Take the compress off the septum of the blood culture vial.
- Without touching anything with the needle, insert it immediately into the septum of the blood culture vial. The blood will be drawn into the vial automatically.
- Once the required amount of blood is in the vial, pull the needle out.
- Immediately place the needle in a sharps collector and properly dispose of the other waste using the usual procedures.
- Gently mix the contents of the vials to blend the blood and the medium. Do not invert the
  vials.
- Take off the nonsterile gloves and throw them with the other waste into the appropriate trash bin.
- Perform hand hygiene.
- Note the volume of blood transferred on the blood culture vial.

#### After collecting the sample

- Clean/disinfect and put away all of the equipment used.
- Perform hand hygiene.
- Place the vials into the specimen transport bag or box.
- Never refrigerate a blood culture vial.
- Follow the usual procedure for transporting samples to the laboratory.



Refer to the SOP in the "Nursing Care Procedures" manual for more details.

# 3.2.4 Collecting dried blood spot (DBS) samples

Some diseases can be diagnosed using a DBS of capillary and/or venous blood. It is important to verify the appropriate sample type, see Chapter 2.

### **Equipment**

Preparing a DBS sample with capillary blood (dengue and measles)

- MODULE DRIED BLOOD SPOT (DBS) & TRANSPORT 2017 [KMEDMSAMDBS3:
  - CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W], 1<sup>st</sup> choice (for other choices, see Chapter 4, Section 4.5.2)
  - HUMIDITY INDICATOR CARD, 10 60 % [ELABHUMI2C-]
  - SILICA GEL, granulated, with saturation indicator, 5 g, bag [SLASSILI1C5]
  - SAFETY LANCET, high flow, blade 1.5 x 1.2 mm, pink, s.u. [STSSLANCSH3]
  - SAMPLE COLLECTION CARD, 5 circles perforated (Munksjö) [STSSSACC2--]
  - (sample collection card) RACK for drying [STSSSACC101]
  - (sample coll card) BAG, plastic, impervious to gas, zip lock [STSSSACC102]
- Additional materials
  - ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
  - SUCROSE, 24% oral solution, 2 mL, vial [SDDCSUCR2V2], for children < 6 months</li>
  - COMPRESS, NON WOVEN, 4 plies, 7.5 cm, non sterile [SDRECOMN7N-]
  - COTTON WOOL, hydrophilic, roll, 500 g [SDRECOTW5R-]
  - CONTAINER, needles/syringes [SINSCONT++]
  - GLOVE, EXAMINATION, latex, s.u. non sterile [SMSUGLOE1+++]

Preparing a DBS sample with venous blood collected in an EDTA tube (dengue, yellow fever, Japanese encephalitis, West Nile fever, Zika, and chikungunya):

- MODULE DRIED BLOOD SPOT (DBS) & TRANSPORT 2017 (voir les détails ci-dessus)
   [KMEDMSAMDBS3]
- Additional materials
  - PIPETTE, AUTOMATIC, adjustable vol. 10-100 μl (Eppendorf) [ELABPIAA0100]
  - (aut.pip.)TIP DUALFILTER, 2-100 μl, yellow rack, ster (Eppdf) [ELABPIATYRF]

See the SOP for collecting a capillary blood or the SOP for collecting venous blood in the "Nursing Care Procedures" manual for more details.

#### Before collecting the sample

- Perform hand hygiene.
- Confirm the patient's identity.

- Explain the procedure to the patient and confirm his medical history, allergies, and history
  of frequent sample collection. Give the patient an opportunity to ask questions, and obtain
  his verbal consent (which should be documented in his chart).
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Perform hand hygiene.
- Assemble the sample collection equipment on a tray or disinfected treatment trolley.

### Sample collection procedure

- Perform hand hygiene and ask the patient to perform hand hygiene with soap and water (if taking the sample from a finger).
- Put on nonsterile gloves
- The DBS sample collection cards must be labelled with the patient's unique identification number and the collection date. Be careful not to touch the circles.
- Choose the puncture site (finger, or side of the heel if child < 6 months). Angle the patient's
  hand downward, palm up, and choose which finger to stick (middle or ring finger). Apply
  intermittent pressure to the chosen finger or the foot.</li>
- For infants (< 6 months), consider giving an oral sucrose solution 2-3 minutes before the stick; if the procedure lasts more than 5 minutes, a second dose can be given. A sheet or towel can be used to hold the child's arms, if necessary.
- Thoroughly disinfect the puncture site with the chlorhexidine wipe, using a back-and-forth motion, for 30 seconds (except in newborns – use warm water and gauze/cotton wool only).
   Let dry.
- Massage around the area to be pricked before and during collection (not on the collection area itself). Do not squeeze the finger/foot.
- Remove the protective cover on the lancet. Hold the finger firmly and place the lancet on the side of the distal phalanx. For heel sticks, flex the patient's foot and hold it in position with your non-dominant hand, placing one finger on the arch and your thumb below the puncture site, at the ankle.
- Press the top of the lancet firmly to prick the puncture site and discard the lancet.
- Wipe away the first drop of blood with a piece of gauze or dry cotton wool and then let the blood flow (ideally it should flow "on its own") onto the circles printed on the sample card.
   The blood should saturate the paper and completely fill the number of circles required by the reference lab.
  - Alternatively, transfer 50 microliters of whole blood using a pipette onto the circles after collecting venous blood (into an EDTA/purple tube).
  - *Note:* If molecular testing is planned, automated pipette tips with filters must be used.
- Apply a compress to the puncture site and press until the bleeding stops.
- Take off the nonsterile gloves and throw them with the other waste into the appropriate trash bins.
- Perform hand hygiene.

### After collecting the sample

 The blood-impregnated sample card should dry naturally, in a horizontal position, for 3 to 4 hours in a place where it is protected from direct sunlight, dust, insects, and draughts. Do not allow filter papers to touch each other, especially before the sample is completely dry. There are drying racks like the rack that comes in the kit.

- Once dry, each DBS card should be stored in an airtight, transparent transport bag with silica gel packets to absorb moisture and a humidity indicator card. The DBS cards should ideally be stored in the cold chain (+2 °C to +8 °C), or at less than 25 °C with no light or humidity, as soon as possible after drying.
- It is important to ensure that DBS cards are completely dry before packaging. Otherwise, test quality may be poor.
- The humidity level of the DBS cards should be checked daily. If the level reaches 30%, the humidity indicator and silica gel should be changed.
- For shipping, the DBS cards should be left in their transport bag with a humidity indicator, but with new silica gel packets. DBS cards are exempt from IATA regulations

# 3.3 Preparing serum

# **Equipment**

- FORCEPS, BRUCELLE, 14 cm, straight, inox [ELABFOBR1--], to remove tubes from the centrifuge
- MARKER, permanent, black, fine point [ELABMARK1B-]
- PIPETTE, TRANSFER, graduated, plastic, sterile, s.u. [ELABPIPT1S-]
- CRYOTUBE, 2.0 mL, conical, ext. thread, sterile DNA/RNAse free [ELABTUMC20EP]
- STORAGE BOX, PP, 9x9 microtubes 1-2 mL, autoclavable [ELABTUMB81PP]
- RACK, PK, 6x4 microtubes, autoclavable [ELABTUMR24PK]
- CENTRIFUGE, hand-operated for 4 tubes 15 mL [ELAECENE1M-], in case electrical centrifuge is not available
- CENTRIFUGE, electrical (Hettich EBA 200), 8 tubes, 230V [ELAECENE9--] and spare parts and electrical protection – see Biomed
- (blds.syst.) TUBE, VACUUM, plastic, SERUM, 2 mL, red [STSSBSVT2S-]
- (blds.syst.) TUBE, VACUUM, plastic, SERUM, 4 mL, red [STSSBSVT4S-]
- After collecting blood in a dry/red tube, leave the tubes on the bench for at least 20 minutes to allow the blood to clot completely before centrifugation. The tubes should then be spun at 1000 g for 10 minutes. With the Hettich EBA 200, this corresponds to about 3200 RPM (revolutions per minute). As with all centrifuges, be sure to balance by placing tubes of equal weight directly opposite each other.
- Manual centrifuges have four slots for tubes. The centrifuge must be well-balanced to prevent damage to the rotor. Manual centrifuges can reach a speed of 3000 RPM.
- If there is no laboratory or no available centrifuge, leave the tube at room temperature for 1 hour and then place it in the refrigerator (+2 °C to +8 °C) in a vertical position until the clot has completely retracted (leaving translucent yellow serum). The sample can be left in the refrigerator for a maximum of 24 hours before separating the serum (for ELISA tests).
- Label a cryotube with the patient's unique identification number and the collection date.
- Transfer the serum into the cryotube with a pipette.
   Notes:

If molecular testing is planned, sterile pipettes or automated pipette tips with filters must be used.

It is important that the sample not be haemolysed, because haemolysis makes analysis impossible. To prevent that, do not transport the sample collection tube before centrifuging it and separating the serum. If that is impossible, reduce the risk of haemolysis by placing the tubes in sponges for transport (to reduce jolting).

# 3.4 Preparing plasma

# **Equipment**

- FORCEPS, BRUCELLE, 14 cm, straight, inox [ELABFOBR1--], to remove tubes from the centrifuge
- MARKER, permanent, black, fine point [ELABMARK1B-]
- PIPETTE, TRANSFER, graduated, plastic, sterile, s.u. [ELABPIPT1S-]
- CRYOTUBE, 2.0 mL, conical, ext. thread, sterile DNA/RNAse free [ELABTUMC20EP]
- STORAGE BOX, PP, 9x9 microtubes 1-2 mL, autoclavable [ELABTUMB81PP]
- RACK, PK, 6x4 microtubes, autoclavable [ELABTUMR24PK]
- CENTRIFUGE, hand-operated for 4 tubes 15 mL [ELAECENE1M-], in case electrical centrifuge is not available
- CENTRIFUGE, electrical (Hettich EBA 200), 8 tubes, 230V [ELAECENE9--] and spare parts and electrical protection see Biomed
- (blds.syst.) TUBE, VACUUM, plastic, K2EDTA, 2 mL, purple [STSSBSVT2E-]
- (blds.syst.) TUBE, VACUUM, plastic, K2EDTA, 4 mL, purple [STSSBSVT5E-]
- (blds.syst.) TUBE, VACUUM, plastic, Li-HEPARIN, 2 mL green [STSSBSVT2HL]
- (blds.syst.) TUBE, VACUUM, plastic, Li-HEPARIN, 4 mL green [STSSBSVT5HL]
- After collecting blood in a tube with anticoagulant (such as: EDTA/purple, heparin/green), thoroughly mix the blood with the anticoagulant by gently and completely inverting the tube 5 to 10 times.
- After that, the tubes can be spun at 1000 g for 10 minutes (or 3200 RPM with the Hettich EBA 200 centrifuge).
- Just like when preparing serum, a manual centrifuge can be used.
- Label a cryotube with the patient's unique identification number and the collection date.
- Transfer the plasma into the cryotube using a sterile pipette.

#### Notes:

- If molecular testing is planned, sterile pipettes or automated pipette tips with filters must be used.
- It is important that the sample not be haemolysed, because haemolysis makes analysis impossible. To prevent that, do not transport the sample collection tube before centrifuging it and separating the plasma. If that is impossible, reduce the risk of haemolysis by placing the tubes in sponges during transport (to reduce jolting).

# 3.5 Collecting ENT and pulmonary samples

# 3.5.1 Nasal/nasopharyngeal aspiration



Pertussis: a surgical mask and gloves are required when handling samples or having any contact with patients.

### **Equipment**

- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- TUBE, SUCTION, conical tip, 50 cm, single use, CH08 [SCTDTUSU08-]
- SYRINGE, s.u., conical tip, 50-60 mL [SCTDSYDF60C]
- CONTAINER, SAMPLE, plast., 60 mL, sterile [STSSCONT6--]
- Explain the procedure to the patient and confirm his medical history, including allergies. Give the patient an opportunity to ask questions, and obtain his verbal consent (which should be documented in his chart).
- Label a sterile specimen container (not the lid) with the patient's unique identification number and the collection date, time, and place.
- Perform hand hygiene and put on gloves.
- Place a few millilitres of sterile normal saline in the nose using a syringe and a thin tube.
- Aspirate the fluid using the tube and syringe.
- Transfer the fluid into the sterile specimen container. Close the container tightly.

# 3.5.2 Collecting nasopharyngeal swabs



Pertussis and diphtheria: a surgical mask and gloves are required when handling samples or having any contact with patients.

COVID-19: A surgical mask, goggles, gown, and gloves are required for sample collection.

- Nasopharyngeal swabbing: pertussis and diphtheria
  - MARKER, permanent, black, fine point [ELABMARK1B-]
  - AMIES AGAR CHARCOAL + SWAB, flocked tip, plast. stick [STSSTRTUAMAC2]
- Nasopharyngeal swabbing: COVID-19
  - MARKER, permanent, black, fine point [ELABMARK1B-]
  - TUBE UTM VIRUS 3 mL + 2 SWABS, flocked tip, plastic stick [STSSTRTUUTM3], 1st choice
  - UNIVERSAL TRANSPORT MEDIUM+DOUBLE SWAB, 3 mL, synth. tip [STSSTRTUUTM1], 2<sup>nd</sup> choice

- Label the tubes with the patient's unique identification number and the collection location, date, and time.
- Explain the procedure to the patient and warn him that he might gag or want to sneeze during the procedure. Confirm his medical history, including allergies. Give the patient an opportunity to ask questions, and obtain his verbal consent (which should be documented in his chart).
- Perform hand hygiene and put on gloves.
- Ask the patient to blow his nose and cough.
- Place the patient in a seated position, asking him to sit on his hands to prevent a defence reflex. Have children sit on a parent's lap and ask the parent to cross their arms over the child's shoulders and to firmly hold the child's hands down.
- Measure the distance from the tip of the patient's earlobe to the base of his nose. Divide
  that distance in half to get the length of swab to insert in the nostril.
- Ask the patient to tip his head back.
- Lift the tip of the nose to open the nostril.
- Without touching the nostril, gently insert the swab into the nostril to the previouslymeasured length, until you feel resistance.
- Keep the shaft of the swab close to the nasal septum, parallel to the palate, for 10 seconds while gently rotating the shaft 5 times to collect the secretions, and then withdraw the swab.
- Place the swab into the appropriate transport medium (see the disease fact sheets in Chapter 2) and swirl the shaft gently in a circular motion. Break off the shaft, if necessary.
- Reclose the cap of the transport medium.
- For diphtheria and pertussis, collect one sample from each nostril. For COVID-19, collect a sample from just one nostril.

# 3.5.3 Collecting oropharyngeal swabs



COVID-19: a surgical mask, goggles, gown, and gloves are required when handling samples or having any contact with patients.

- MARKER, permanent, black, fine point [ELABMARK1B-]
- DEPRESSOR, TONGUE, wooden [SMSUDEPT1W-]
- TUBE UTM VIRUS 3 mL + 2 SWABS, flocked tip, plastic stick[STSSTRTUUTM3], 1st choice
- UNIVERSAL TRANSPORT MEDIUM+DOUBLE SWAB, 3 mL, synth. tip [STSSTRTUUTM1], 2<sup>nd</sup> choice
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Label the tubes with the patient's unique identification number and the collection location, date, and time.
- Perform hand hygiene and put on gloves.
- Insert the swab into the patient's mouth.
- Swab the back of the throat and the tonsils (avoid the tongue).
- Place the tip of the swab in the same viral transport medium as the nasopharyngeal swab, break off the upper part of the shaft, and screw the cap closed.

# 3.5.4 Collecting throat swabs



Diphtheria: a surgical mask and gloves are required when handling samples or having any contact with patients.

### **Equipment**

- MARKER, permanent, black, fine point [ELABMARK1B-]
- DEPRESSOR, TONGUE, wooden [SMSUDEPT1W-]
- AMIES AGAR CHARCOAL + SWAB, flocked tip, plast. stick [STSSTRTUAMAC2]



Do not do collect this kind of sample after the patient has eaten or received oral care.

- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Label the tubes with the patient's unique identification number and the collection location, date, and time.
- Perform hand hygiene and put on gloves.
- Get rid of as much salivary contamination as possible.
- Depress the tongue with the tongue depressor to get a good view of the oropharynx and tonsils.
- Ask the patient to say, "AAHHH". Making that sound helps suppress the gag reflex.
- Identify any inflamed, purulent (soft white coating), necrotic, or ulcerated areas and pseudomembranes.
  - Rub the swab over the surface of each tonsil, the pharyngeal mucosa, the faucial pillars, and any pathological-looking surface (avoid touching the tongue, the uvula, and the posterior pharyngeal wall). Ideally, **only someone with experience in collecting this type of sample** should delicately rub the pseudomembranes and the tissues beneath them. But be careful not to detach them; be extremely gentle when collecting the sample. If there is exudate or ulceration, sample it.
- If a defence reflex by the patient results in the swab making contact with any other surface, redo the sample.
- Next, place the swab into a tube containing Amies transport medium with charcoal.
- Close the tube tightly.

# 3.5.5 Collecting buccal swabs in viral transport medium for suspected filovirus

For investigating suspected Ebola virus disease deaths in the community or when it is impossible to sample a patient – even using capillary blood.



Whenever a haemorrhagic fever is suspected, personal protective equipment is essential, two people are needed for sample collection, and samples must be triple-packaged. Suspected cases are always placed in an isolation zone.

- MARKER, permanent, black, fine point [ELABMARK1B-]
- BAG, plastic, 14 x 17 cm, re-sealable zipper [SMSUBAGP14-] or transport tube transmitted by the laboratory

- UNIVERSAL TRANSPORT MEDIUM+DOUBLE SWAB, 3 mL, synth. tip [STSSTRTUUTM1], 2<sup>nd</sup> choice
- TUBE UTM VIRUS 3 mL + 2 SWABS, flocked tip, plastic stick [STSSTRTUUTM3], 1st choice
- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]
- CHLORINE SPRAYER (Birchmeier) 15 L, backpack [CWATSPRAB5-]

### Preparation before entering the isolation zone

- On a disinfected tray, assemble the sample collection equipment (see list above), as well as:
  - A plastic bucket or container with 0.5% chlorine solution for transport
  - A spray bottle with 0.5% chlorine solution (for decontamination or accidental exposure)
- Label the specimen transport bags/tubes, transport medium, and lab request forms with the patient's unique identification number, the date, and the time.
- Put on the appropriate personal protective equipment.

#### In the isolation zone

- Enter in pairs (a medical person and a hygienist).
  - The hygienist sprays the body fluids, if any, around the body. Do not put chlorine on the body.
  - The collector swabs the upper and lower gums using enough pressure and friction to collect saliva and epithelial cells.
- Place the swab into the viral transport medium tube (1° packaging). Break off the end of the handle and close the tube completely. Place the tube in a plastic bag (2° packaging) with an absorbent material.
- The hygienist then sprays the collector's gloved hands and the outside of the plastic bag with 0.5% chlorine solution.

## Transporting the sample out of the isolation zone

- The collector transfers the sample in its transport container or another plastic bag (3o packaging) out of the isolation zone by going "over the barrier" (this can be done in the undressing zone) without touching anything.
- The inside of the 3<sup>rd</sup> packaging is sprayed with 0.5% chlorine solution and closed for transfer to the laboratory (if nearby) or placed in a UN 2814 transport box (if the lab is far away or international – 4<sup>th</sup> packaging). That container can be carried without gloves

# 3.5.6 Collecting sputum samples



Pertussis: a surgical mask and gloves are required when handling samples or having any contact with patients.

- MARKER, permanent, black, fine point [ELABMARK1B-]
- CONTAINER, SAMPLE, plast., 60 mL, sterile [STSSCONT6--]

- Explain the procedure to the patient and confirm his medical history, including allergies. Give the patient an opportunity to ask questions, and obtain his verbal consent (which should be documented in his chart).
- Label the sputum container (not the lid) with the patient's unique identification number and the collection date, time, and location.
- Ask the patient to collect the sample outside.
- Ask the patient to wash his hands and rinse his mouth out with clean water and, if possible, to brush his teeth.
- Ask the patient not to open the sputum container except when collecting the sample.
- Explain to the patient the steps for collecting the sample, as follows:
  - Take a deep breath and hold it in for 5 seconds.
  - Release the air slowly.
  - Do the same thing a second time (deep breath, hold for 5 seconds, and release slowly).
  - Then start by taking another deep breath, and then cough until there is sputum in your mouth.
  - Spit into the sputum container.
- Ask the patient to produce about 5 mL or the equivalent of a teaspoon.
- Ask the patient to close the lid well and wash his hands before bringing the sample to the laboratory.

# 3.5.7 Collecting sputum samples and swabs for plague



A high-filtration (FFP2/N95) mask, gown, goggles, and gloves are required when handling samples or having any contact with patients.

Two people are required for collecting sputum samples for suspected plague.

#### Equipment

- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]
- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1], in case Cary Blair® is not available
- MARKER, permanent, black, fine point [ELABMARK1B-]
- CONTAINER, SAMPLE, plast., 60 mL, sterile [STSSCONT6--]
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1], depending of availability. If Cary Blair® is not available, use sterile physiological serum or sterile water to soak the swab
- PLAIN TUBE+SWAB, flocked polyester tip, plastic, st. [STSSTRTUP4-], in case Cary Blair® is not available
- BAG, plastic, 14 x 17 cm, re-sealable zipper [SMSUBAGP14-] or CONTAINER, PROTECTION, transport of sample, plastic [STSSCONP++]

# **Preparing for sample collection**

- This sample is collected by two people: the collector, who is in the room with the patient and helps the patient, and the assistant, who waits outside to receive the sample and helps the collector remove his personal protective equipment.

- Prepare a trash bag for waste to be destroyed, a bag for reusable waste to be disinfected, and a sharps collector.
- Prepare the 0.5% chlorine solution.
- Take the Cary-Blair® transport medium out of the refrigerator 30 minutes before inoculation
  if stored in the cold chain.
- Label the sputum container and the Cary-Blair® medium (patient's unique identification number and the collection location, date and time) and prepare all of the sample collection equipment.
- Wear a gown, an N95 mask, sterile gloves (over the gown), goggles, and closed shoes (see Chapter 4).
- Prevent draughts in the sample collection room (no fans or open windows while the sample is being collected).
- Confirm the patient's identity.
- Explain the procedure to the patient and confirm his medical history and allergies. Give the
  patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Explain to the patient what a sputum sample is and the steps for collecting it:
  - He will have to produce about 5 mL, or the equivalent of a teaspoon, of sputum. If the quality of the sample is good, the amount can be smaller.
  - Sputum is thick and mucoid, comes from the lungs, and may be whitish, green, or tinged with blood.
  - Saliva and nasal secretions are not good samples (clear and liquid).

## Steps for collecting the sample

- Ask the patient to rinse his mouth with clean water.
- Hand the patient the sputum container and ask him not to touch the inside.
- Position the patient and stand behind him to avoid any exposure when the patient coughs.
- Instruct the patient as follows:
  - Take a deep breath and hold it for 5 seconds. Release the air slowly. Do this twice.
  - The third time, take a deep breath, hold the breath for a few seconds, and then blow out hard.
  - Bring the sputum container close to your mouth and blow hard again. (This movement will help bring the sputum up from the lungs to the mouth.) **Do not touch the sputum container with your mouth.**
  - Spit directly into the sputum container.
- Check the quality of the sputum. If the quality is not good, ask the assistant for a new sputum container and repeat the procedure.
- Close the sputum container tightly for shipping as is, or insert a swab into the container and sample the mucosanguineous parts. Make sure the swab is well-saturated.
- Then push the swab into the Cary-Blair® agar.
  - If Cary-Blair® transport medium is unavailable, you can use a swab soaked in sterile normal saline or sterile water to transport the sample. Add enough sterile saline/sterile water to ensure that the swab remains immersed in it while in transit and does not dry out.
- Close the Cary-Blair<sup>®</sup> tube tightly.
- Take the Cary-Blair® tube and clean the outside with paper towel to remove any trace of sputum. Do the same with the sputum container if sending the sputum container itself.
- Throw all materials that came in contact with the patient and the remaining sputum into the bag for waste to be destroyed.

- Protect the Cary-Blair® tube or sputum container by wrapping it in paper towel.
- Call the assistant outside the room/at the door. The assistant should not enter the room.
   He should wear gloves and have a specimen transport bag/container ready.
- Place the paper towel-wrapped Cary Blair® tube or sputum container into the specimen transport bag/container. Do not touch the specimen transport bag/container when transferring the sample.
- The assistant closes the bag/container and disinfects the outside with 0.5% chlorine solution.
- The assistant removes his gloves and washes his hands.
- The collector removes his personal protective equipment, beginning with his gloves, and washes his hands; he then removes his gown and washes his hands; he takes off his goggles and washes his hands; and finally, he removes his mask and washes his hands.
- A third, UN 3373, container is required for transport to an outside laboratory.

# 3.6 Collecting a stool sample

#### 3.6.1 Stool

✓ Polio, hepatitis E, and cholera

# **Equipment**

- MARKER, permanent, black, fine point [ELABMARK1B-]
- CONTAINER, SAMPLE, plast., 60 mL, non sterile, urine [STSSCONT6U-]
- CONTAINER, SAMPLE, PP, 120-125 mL, sterile, stools+spatula [STSSCONT12SSS] only for poliomyelitis

For hospitalised or incontinent patients, add:

- BEDPAN, with handle, polypropylene [EMEQBEDP1P-]
- NAPPY adult, medium or large size, super absorption, single use [PHYPDIAPLAA or PHYPDIAPMAA]

#### Hospitalised patient wearing diapers

- Label a specimen container with the patient's unique identification number and the collection date, time, and place.
- Explain the procedure to the patient and confirm his medical history and allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should
  be documented in his chart). Give the patient the specimen container and tell him how to
  collect the stool:
  - In case of loose stool: collect directly in the container.
  - In case of soft or formed stool:
    - Either collect directly in the container (sterile)
    - Or, using the sterile spatula inserted in the lid of the sterile stool container, place a piece of stool the size of the proximal phalanx of an adult thumb or a walnut in the vial (collect it from the centre of the stool). If the stool contains pus or blood, sample those parts in particular.
  - Warn the patient not to contaminate the stool with urine, toilet paper, soap, etc.
- Do not fill the container to the brim.
- Screw the container lid tightly closed.

# Hospitalised patient wearing diapers

- Label a specimen container with the patient's unique identification number and the collection date, time, and place.
- Explain the procedure to the patient and confirm his medical history and allergies. Give the
  patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Perform hand hygiene and put on gloves.
- Immediately transfer as much stool as possible into the container using the spatula.
   Note: If stools are totally liquid, collect a rectal swab
- Do not fill the container to the brim.
- Screw the container lid tightly closed.
- Take off the gloves and perform hand hygiene

### **Continent hospitalised patient**

- Label a specimen container with the patient's unique identification number and the collection date, time, and place.
- Explain the procedure to the patient and confirm his medical history and allergies. Give the
  patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Give the patient a disinfected bedpan and ask him to defecate directly into the bedpan, either in bed or in the latrine.
- Warn the patient not to contaminate the stool with urine, toilet paper, soap, etc.
- Perform hand hygiene and put on gloves when collecting the bedpan.
- Place a piece of stool the size of the proximal phalanx of an adult thumb or a walnut into the
  vial (collect it from the centre of the stool). If the stool contains pus or blood, sample those
  parts in particular.
- Do not fill the container to the brim.
- Screw the container lid tightly closed.
- Take off the gloves and perform hand hygiene.

# 3.6.2 Collecting a faecal swab in transport medium

✓ Cholera, shigellosis, and hepatitis E

- MARKER, permanent, black, fine point [ELABMARK1B-]
- CONTAINER, SAMPLE, plast., 60 mL, non sterile, urine [STSSCONT6U-]
- AMIES AGAR CHARCOAL + SWAB, flocked tip, plast. stick [STSSTRTUAMAC2], for shigellosis (1st choice)
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1] for Cholera, Shigellosis
- UNIVERSAL TRANSPORT MEDIUM+DOUBLE SWAB, 3 mL, synth. tip [STSSTRTUUTM1--], for Hepatitis E
- Take the transport medium out of the refrigerator 30 minutes before inoculation if stored in the cold chain.
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Label the specimen container, give it to the patient and tell him how to collect the stool (see procedure for collecting stool samples, (see chapter3, Section 3.6.1).
- Label the transport medium tube (patient's unique identification number and the collection location, date, and time).
- Insert the swab from the transport medium into the container holding the stool. If shigellosis
  is suspected, sample the mucosanguineous parts. Make sure the swab is well-saturated.
- Next, place the swab into the transport medium agar (Amies with charcoal or Cary-Blair®) or into the liquid viral transport medium.
- Break off the plastic shaft at the indicated location.
- Close the tube tightly.
- Protect the sample from excessive heat (< 25 °C) and direct sunlight by placing it in a protective container or aluminium foil.

# 3.6.3 Collecting a stool sample on filter paper

#### ✓ Cholera

#### Equipment

#### MODULE, SAMPLE, 001, transport [KMEDSAM1C-]

- FORCEPS, BRUCELLE, 14 cm, straight, inox [ELABFOBR1--]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- − FILTER PAPER, DISK, not impregnated, Ø 6 mm [ELABPAPFD6-]
- CRYOTUBE, 2.0 mL, round, ext. thread, non-sterile [ELABTUMC20EN]
- PHYSIOLOGICAL SALINE SOLUTION, NaCl, 0.9%, 5 mL, plas. vial [SLASSODC9B5]
- BAG, plastic, 10 cm x 10 cm, re-sealable zipper [SMSUBAGP10-]
- BAG, plastic, for health card, 16x22 cm, re-sealable zipper [SMSUBAGP16-]
- DEPRESSOR, TONGUE, wooden [SMSUDEPT1W-]
- CONTAINER, SAMPLE, plast., 60 mL, non sterile, urine [STSSCONT6U-]
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1]
- Explain the procedure to the patient and confirm his medical history and allergies. Give the
  patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Give the patient the specimen container and tell him how to collect the stool (see procedure for collecting stool samples, Chapter 3, Section 3.6.1).
- Label the tube already containing a filter paper disk (patient's unique identification number and the collection location, date, and time).
- Open the tube.
- Pick up the disk with clean forceps or a sterile needle without touching the edges of the tube.
- To prevent contamination of subsequent samples:
  - Disinfect and wash the forceps between samples (hold the forceps in a flame and let cool, or disinfect with a chlorine solution and rinse well with filtered water).
  - Use a new sterile needle for each sample.
- Soak the disk in the stool sample and make sure it is saturated.
- Place the saturated disk back in the tube.
- Add 2 to 3 drops of normal saline to prevent the sample from drying out.
   Close the tube completely.

*Note:* collect the sample before starting antibiotics, and do not sample stool that has been chlorinated.

# 3.6.4 Collecting a rectal swab in transport medium

Cholera and shigellosis

- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1]
- WATER for injection, 10 mL, plastic amp. [DINJWATE1A-]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- AMIES AGAR CHARCOAL + SWAB, flocked tip, plast. stick [STSSTRTUAMAC2], for shigellosis (1st choice)
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1]

- Take the transport medium out of the refrigerator 30 minutes before inoculation if stored in the cold chain.
- Label the tube (patient's unique identification number and the collection location, date, and time).
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Perform hand hygiene and put on gloves.
- Moisten the swab with sterile water or sterile normal saline.
- Swab the rectum, scraping the mucosa well. The swab must be stained with stool.
- Then push the swab into the Cary-Blair® or Amies with charcoal agar.
- Close the tube tightly. Store the sample at +15 °C to +25 °C and protect it from direct sunlight by putting it into a protective container or wrapping it in aluminium foil.

# 3.7 Collecting a urine sample

✓ Leptospirosis and arbovirosis

- MARKER, permanent, black, fine point [ELABMARK1B-]
- CONTAINER, SAMPLE, plast., 60 mL, sterile [STSSCONT6--] Notes:
  - This equipment is for use with ambulatory patients
  - For hospitalised patients, refer to the SOP on collecting urine with a urinary catheter or bag in the Nursing Care Procedures manual.
- Label the container (sterile cup/vial) with the patient's unique identification number and the collection location, date, and time).
- Explain the procedure to the patient and confirm his medical history and allergies. Give the
  patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Ask the patient to wash his hands.
- Ask the patient not to open the vial until the last minute or touch the inside.
- If possible, collect the first morning urine. If that is not possible, wait a while (ideally 4 hours) after last urination to collect the sample.
- The patient should clean the urinary area before collecting the sample with soap and water,
   and then disinfect the urinary meatus and let it dry.
- If the patient is able to do so, he should begin urinating in the toilet, interrupt the flow of urine, and then collect the second stream in the specimen cup. Once the sample has been collected, the patient can finish urinating directly into the toilet.
- Close the container tightly.
- Ask the patient to wash his hands.

# 3.8 Collecting a skin or skin lesion sample

#### 3.8.1 Skin or skin lesion swab

✓ Diphtheria

## **Equipment**

- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- AMIES AGAR CHARCOAL + SWAB, flocked tip, plast. stick [STSSTRTUAMAC2]
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Label the transport medium (patient's unique identification number and the collection location, date, and time).
- Perform hand hygiene and put on gloves.
- Clean the lesions with sterile normal saline.
- Swab the periphery of the pseudomembranes covering the ulceration.
- Insert the swab into Amies transport medium with charcoal.

# 3.8.2 Swabbing lesion fluid from vesicles



Poxvirus: if monkeypox is suspected, appropriate personal protective equipment (gloves, gown, FFP2/N95 respirator, and face mask/goggles) is required when handling samples or having any contact with patients.

- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- UNIVERSAL TRANSPORT MEDIUM+DOUBLE SWAB, 3 mL, synth. tip [STSSTRTUUTM1--], depending of the reference laboratory
- PLAIN TUBE+SWAB, flocked polyester tip, plastic, st. [STSSTRTUP4-]
- Label the tube (patient's unique identification number and the collection location, date, and time).
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Perform hand hygiene and put on personal protective equipment.
- Pierce the vesicle with the swab and swab the base of the lesion by rotating the sterile swab to collect the fluid. If the lesion is dry, wet the swab with a drop of sterile normal saline beforehand.

- Place the swab into the tube.
- Use viral transport medium as recommended by the reference laboratory. It is not always
- Break off the plastic shaft at the location indicated, if applicable, and reclose the tube.
- The base of the lesion can also be swabbed with a sterile swab after pustules collapse.

# 3.8.3 Collecting scab samples



Poxvirus: if monkeypox is suspected, appropriate personal protective equipment (gloves, gown, FFP2/N95 respirator, and face mask/goggles) is required when handling samples or having any contact with patients.

- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- CRYOTUBE, 2.0 mL, conical, ext. thread, sterile DNA/RNAse free [ELABTUMC20EP]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- NEEDLE, s.u., Luer, 26G (0.45 x 13 mm), brown, ID [SINSNEED26-]
- Label the cryotubes (patient's unique identification number and the collection location, date, and time).
- Explain the procedure to the patient and confirm his medical history, including allergies. Give the patient an opportunity to ask questions, and obtain his verbal consent (which should be documented in his chart).
- Perform hand hygiene. Put on personal protective equipment.
- Clean the lesion with the antiseptic wipe (see Chapter 4, for other options) and let dry.
- Using a sterile 26G needle, collect at least four scabs: take two scabs from each of two different parts of the body.
- Place each scab in its own cryotube. Do not add viral transport medium. The scabs must stay completely dry.
- Close the cryotubes tightly.

# 3.9 Plague bubo

# 3.9.1 Plague bubo puncture



Sample collection technique **to be performed by a trained doctor or nurse**.

A high-filtration (FFP2/N95) mask, gown, goggles, and gloves are required when handling samples or having any contact with patients. Sample collection requires two people.

- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]
- ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
- POLYVIDONE IODINE, 10%, solution (100 mL, 200 mL, fl. dropper bottle or 500 mL)
   [DEXTIODP1S-]
- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- STERILE DRAPE, non-woven [ELINDRSS++]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- CONTAINER needles/syringes [SINSCONT+++]
- NEEDLE, s.u., Luer, 19G (1.1 x 40 mm) cream, IV [SINSNEED19-]
- SYRINGE, s.u., Luer, 5 mL [SINSSYDL05-]
- GLOVES, SURGICAL, latex, s.u., sterile, pair, 7.5 [SMSUGLOS75-]
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1]
- PLAIN TUBE+SWAB, flocked polyester tip, plastic, st. [STSSTRTUP4-], in case Cary Blair<sup>®</sup> is not available
- BAG, plastic, 14 x 17 cm, re-sealable zipper [SMSUBAGP14-], or CONTAINER, PROTECTION, transport of sample, plastic [STSSCONP++]
- Allow Cary-Blair® transport medium to come to room temperature for about 30 minutes if it was stored in the cold chain.
- Collecting this sample requires two people: the collector, who is in the room with the patient
  and performs the procedure, and the assistant, who waits outside to receive the sample and
  helps the collector remove his personal protective equipment.
- Prepare a trash bag for waste to be destroyed, a bag for reusable waste to be disinfected, and a sharps collector.
- Prepare a 0.5% chlorine solution.
- Label the Cary-Blair<sup>®</sup> (patient's unique identification number and the collection location, date and time) and prepare all of the sample collection equipment.
- Wear a gown, a respirator (FFP2/N95), sterile gloves (over the gown), goggles, and closed shoes. Use a sterile drape.
- Confirm the patient's identity.
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Disinfect the bubo and the area around it with povidone-iodine and let dry.
- Immobilise the bubo with your hand.

- With a 5-mL syringe and a 19G needle, draw 2 mL of sterile normal saline and then stick the bubo perpendicularly, inject and aspirate 1 to 2 mL of normal saline several times in the bubo. Collect at least 2 mL of pus/fluid. Withdraw the needle and syringe.
- Soak a sterile swab in the contents of the syringe without detaching the needle.
- Insert the swab into the Cary-Blair® medium.
  - If Cary-Blair® transport medium is not available, you can use a swab soaked in normal saline or sterile water to transport the sample. Add enough sterile normal saline/water to ensure that the swab remains immersed while in transit and does not dry out.
- Place the Cary-Blair®/tube on the rack.
- Throw the needle into the sharps collector (if there is no needle removal system, throw in both the needle and syringe without disconnecting them). Throw the used equipment in the bag for destruction or disinfection.
- Place a piece of medical tape over the puncture site.
- Take the Cary-Blair® tube and clean the outside with paper towel.
- Throw any materials that came in contact with the patient and the pus in the bag for destruction.
- Protect the Cary-Blair® tube by wrapping it in paper towel.
- Call the assistant outside the room/at the door. The assistant should not enter the room. He should wear gloves and have a transport bag/container ready.
- Place the tube with the sample, wrapped in paper towel, into the transport bag/container.
   Do not touch the bag during the transfer.
- The assistant closes the transport bag/container and disinfects the outside with 0.5% chlorine solution.
- The assistant removes his gloves and washes his hands.
- The collector removes his personal protective equipment, beginning with his gloves, and washes his hands; he then removes his gown and washes his hands; he takes off his goggles and washes his hands; and finally, he removes his mask and washes his hands.

# 3.9.2 Collecting pus from an oozing plague bubo



A high-filtration (FFP2/N95) mask, gown, goggles, and gloves are required when handling samples or having any contact with patients. Two people are needed for collecting this sample.

- POLYVIDONE IODINE, 10%, solution (100 mL, 200 mL, fl. dropper bottle or 500 mL)
   [DEXTIODP1S-]
- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]
- SODIUM chloride, 0.9%, 10 mL, plastic amp. [DINJSODC9AP1], if Cary-Blair® not available
- MARKER, permanent, black, fine point [ELABMARK1B-]
- STERILE DRAPE, non-woven [ELINDRSS++]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- GLOVES, SURGICAL, latex, s.u., sterile, pair, 7.5 [SMSUGLOS75-]
- CARY-BLAIR GEL+ SWAB, flocked tip, plast stick [STSSTRTUCB1]
- PLAIN TUBE+SWAB, flocked polyester tip, plastic, st. [STSSTRTUP4-], if Cary-Blair® is not available
- BAG, plastic, 14 x 17 cm, re-sealable zipper [SMSUBAGP14-], or CONTAINER, PROTECTION, transport of sample, plastic [STSSCONP++]

- Allow the Cary-Blair® transport medium to come to room temperature for about 30 minutes
  if it was stored in the cold chain.
- Collecting this sample requires two people: the collector, who is in the room with the patient
  and performs the procedure, and the assistant, who waits outside to receive the sample and
  helps the collector remove his personal protective equipment.
- Prepare a trash bag for waste to be destroyed, a bag for reusable waster to be disinfected, and a sharps collector.
- Prepare a 0.5% chlorine solution.
- Label the Cary-Blair® (patient's unique identification number and the collection location, date and time) and prepare all of the sample collection equipment.
- Wear a gown, a respirator (N95), sterile gloves (over the gown), goggles, and closed shoes.
- Confirm the patient's identity.
- Explain the procedure to the patient and confirm his medical history, including allergies. Give
  the patient an opportunity to ask questions, and obtain his verbal consent (which should be
  documented in his chart).
- Disinfect the bubo and the area around it with povidone-iodine and let dry.
- Soak a sterile swab in the fluid oozing from the bubo.
- Insert the swab into the Cary-Blair® medium.
  - If Cary-Blair® transport medium is not available, you can use a swab soaked in normal saline or sterile water to collect and transport the sample. Add enough sterile normal saline/water to ensure that the swab remains immersed while in transit and does not dry out.
- Place the Cary-Blair®/tube on the rack.
- Place medical tape over the collection site.
- Take the Cary-Blair®/tube and clean the outside with paper towel.
- Throw any materials that came in contact with the patient or the pus in the bag for destruction.
- Protect the Cary-Blair<sup>®</sup>/tube by wrapping it in paper towel.
- Call the assistant outside the room/at the door. The assistant should not enter the room. He should wear gloves and have a transport bag/container ready.
- Place the tube with the sample, wrapped in paper towel, into the transport bag. Do not touch the specimen transport bag/container when transferring the sample.
- The assistant closes the transport bag/container and disinfects the outside with 0.5% chlorine solution.
- The assistant removes his gloves and washes his hands.
- The collector removes his personal protective equipment, beginning with his gloves, and washes his hands; he then removes his gown and washes his hands; he takes off his goggles and washes his hands; and finally, he removes his mask and washes his hands.

# 3.10 Cerebrospinal fluid

# 3.10.1 Collecting CSF using lumbar puncture (technique to be performed by a doctor or a nurse trained in the technique)

# **Equipment**

# (laboratory module) MENINGITIS, lumbar puncture, 25 tests [KMEDMLAB118]:

- POLYVIDONE IODINE, 10%, solution (100 mL, 200 mL, fl. dropper bottle or 500 mL)
   [DEXTIODP1S-]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- (tube Ø 13/15 mm, 5 mL) RACK [ELABTUBE12R]
- TUBE, CENTRIFUGE, 15 mL, conical bottom, sterile PS crystal [ELABTUCE1SPC]
- TUBE, CENTRIFUGE, 15 mL, conical bottom, sterile PP [ELABTUCE1SPP], if PASTOREX
- (tube, centrifuge, 15 mL, Ø 18 mm) RACK [ELABTUCER1-]
- KIDNEY DISH, 26 cm x 14 cm, stainless steel [EMEQKIDD26-]
- TRAY, DRESSING, 30 x 20 x 3 cm, stainless steel [EMEQTRAD3--]
- FORCEPS, HAEMOST. KOCHER, 14 cm, 1x2 teeth curved, 16-13-14 [ESURFOAK14C]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- ADHESIVE TAPE, fabric, 2-2.5 cm x 5 m [SDRETAPA025]
- CONTAINER needles/syringes [SINSCONT+++]
- NEEDLE, SPINAL L.P., Luer, s.u., 20G (0.9 x 90 mm) [SINSNESD20-]
- NEEDLE, SPINAL L.P., Luer, s.u., 22G (0.7 x 40 mm) [SINSNESD22-]
- GLOVES, SURGICAL, latex, s.u., sterile, pair, 7.5 [SMSUGLOS75-]

## Additional materials:

- ALCOHOL-BASED HAND RUB, solution/gel, 500 mL, bot. [DEXTALCOSS-]
- MASK, SURGICAL, IIR type, s.u. [ELINMASS3--]
- STERILE DRAPE, non-woven [ELINDRSS++]
- Perform hand hygiene.
- Confirm the patient's identity and explain the procedure to him. Obtain his verbal consent (and document it in the chart).
- Ask the patient to empty his bladder/bowels before starting the procedure.
- Ensure patient privacy (safe, quiet room with a curtain or screen, if necessary).
- Clean/disinfect the tray.
- Perform hand hygiene.
- Prepare the equipment on the tray.
- Label the tubes with the patient's unique identification number and the collection date, time, and location:
  - 1 tube for each Trans-Isolate (T-I);
  - 1 tube for PCR and additional analyses;
  - 1 special tube for Pastorex®, if it is done (special tube that can tolerate a temperature of 100 °C: PPa)

a PP: polypropylene

- Perform hand hygiene.
- For children less than 6 months of age, administer oral sucrose solution.
- Perform hand hygiene.
- Position the patient, explaining the position needed:
  - Sitting: On the edge of the bed, body bent forward, back rounded, chin to chest, chest supported by a pillow. If the patient is a child, hold him tightly to prevent him from moving during the puncture. Make sure that the airways are clear and the child can breathe normally.
  - Lying down (if the patient is unconscious/a young infant): Lay the patient down on his side on the edge of the bed, back rounded (foetal position), chin to chest, knees raised toward the chest, head resting on a pillow. The patient should be on a firm surface so that the spinal column is parallel to the surface and the transverse axis of the back is vertical. Be especially careful with young infants. The assistant should not hold a young infant by the neck or flex the neck, to avoid obstructing the airways.
  - Newborns must be kept as warm as possible and not undressed until the moment of the puncture.
- The assistant helps the doctor put on his personal protective equipment, if necessary.
- Identify the anatomical landmarks
  - Locate the space between the third and fourth (L3-L4) or fourth and fifth (L4-L5) lumbar vertebrae. (L3 is located where a line drawn between the iliac crests crosses the spinal column.)

Nurse	Doctor or trained nurse	
Wash hands or disinfect them with an alco-hol-based solution.	Perform hand hygiene. Put on sterile gloves.	
Clean a wide area around the puncture site with antiseptic solution or ordinary soap. Rinse and dry the skin.		
Place a drape under the puncture area.		
Open a bag of sterile compresses and soak them in a skin-safe 10% povidone-iodine so-lution.		
Open another bag of sterile compresses, soak them in a skin-safe 10% povidone-iodine so-lution, and hand them to the doctor.	Swab a wide area around the puncture site a second time with antiseptic. A local anaesthetic (lidocaine 1%) may be administered by infiltration into the skin around the puncture site.	
Ask the patient to stay still and breathe calmly to prevent injury.		
Instruct the patient to report any sudden pain, burning, or other sensation.		

Nurse	Doctor or trained nurse
Open the bag with the spinal needle and hand it to the doctor.  Face the patient so you can monitor him. Help babies stay in position by placing one hand behind their head and the other behind their knees.	Take the spinal needle using aseptic technique.  Perform the lumbar puncture, generally at the level of the iliac crests:  Use a spinal needle fitted with a stylet (22G for a young infant and 20G for an older infant or child; if unavailable, you can use a hypodermic needle).  Insert the needle in the middle of the intervertebral space, aiming toward the umbilicus.  Slowly advance the needle. It will penetrate easily until it reaches the ligament between the spinous processes (interspinous ligament). It will take a bit more pressure to penetrate this ligament, and then you will feel less resistance when penetrating the dura mater. That decreased resistance is not always felt with young infants, so advance the needle very carefully.  Withdraw the stylet; some drops of cerebrospinal fluid will exit the needle. If that does not happen, you can reinsert the stylet and advance the needle a bit farther.  Open the tubes and place them one after another under the needle, letting the necessary amount of CSF flow into each tube. Immediately close the tubes using aseptic technique:  20 drops (1 mL) for each TI;  40 drops (2 mL) for PCR and additional analyses;  20 drops (1 mL) in a special PP tube for Pastorex®, if done.  Reinsert the stylet and then withdraw the needle and stylet completely, applying pressure to the puncture site for a few seconds. Cover with a sterile dressing.  Advancing the needle too far can puncture the lumbar vein. That will cause a "traumatic puncture" and the cerebrospinal fluid will be tinged with blood. Should that happen, withdraw the needle and start again in a different intervertebral space.
Soak a sterile compress with antiseptic and hand it to the doctor.	After withdrawing the needle, put pressure on the puncture site with the antiseptic-soaked compress.  Discard the needle into the sharps collector.
Hand the doctor a dry sterile compress.	Apply the dry compress to the puncture site.
Apply medical tape.	

- Have conscious patients lay on their back or side, depending on their preference; lay unconscious patients on their side.
  - Ask patients to continue lying down for 2 hours after the lumbar puncture to prevent headache.
  - Place a urinal or bedpan within reach.
- Dispose of the waste.
- Remove personal protective equipment.
- Clean and disinfect the tray.
- Wash hands or disinfect them with an alcohol-based solution.
- Finish entering the data in the patient's chart.
- Monitor the patient.

# 3.10.2 Inoculating CSF into Trans-Isolate (T-I) medium

# **Equipment**

# (laboratory module) TRANSPORT MEDIUM FOR CSF 2019 [KMEDMLAB1201]

- NEEDLE, sAIGUILLE, u.u., Luer, 19G (1.1 x 40 mm) cream, IV [SINSNEED19-]
- NEEDLE, sAIGUILLE, u.u., Luer, 21G (0.8 x 40 mm) green, IM [SINSNEED21-]
- SYRINGE, sSERINGUE, u.u., Luer, 1 mL, graduated 1/100 [SINSSYDL01-]
- CONTAINER, PROTECTION, transport of sample, plastic, Ø 44 mm [STSSCONP044P]

# (lab module) TRANSPORT MEDIUM FOR CSF cold chain 2019 [KMEDMLAB1201B]

- TRANSPORT MEDIUM FOR CSF (Trans-Isolate) [STSSTRTUTI1]

#### Additional materials:

- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W]
- POLYVIDONE IODINE, 10%, solution (100 mL, 200 mL, fl. dropper bottle or 500 mL)
   [DEXTIODP1S-]
- MARKER, permanent, black, fine point [ELABMARK1B-]
- COMPRESS, GAUZE, 10 cm, 12 plies, 17 threads, sterile [SDRECOMP1S-]
- COTTON WOOL, hydrophilic, roll, 500 g [SDRECOTW5R-], in case the sending is postponed
- CONTAINER, needles/syringes [SINSCONT+++]

### Perform the lumbar puncture

- Prepare two T-I bottles per patient: one to send to the international reference lab (in Oslo) and one that will stay in the country (national reference lab). If the samples are sent to only one lab, it is unnecessary to inoculate two T-I bottles.
- Leave the T-I at room temperature for 30 minutes.
- Check to make sure that there is no bacterial growth in the solid phase and/or that the liquid phase is not cloudy.
- Label the bottles with the patient's unique identification number and the collection date, time, and place.
- Lift the metal cover in the centre and disinfect the rubber stopper with chlorhexidine or povidone-iodine. Let dry.
- Draw 0.5 to 1 mL of CSF with a sterile syringe and a 21G needle.
- Inject the CSF into the T-I through the rubber stopper.
- Throw the needle and syringe into the sharps collector.

- If the T-I cannot be sent to the lab that day, it must be ventilated for at least 24 hours by sticking a sterile 19G needle (plugged with cotton wool) through the T-I's rubber stopper. T-I can be stored, ventilated, for no more than 2 to 3 weeks. The T-I absolutely must be stored at room temperature after inoculation (at or below 40 °C).
  - *Note:* the needle must not be immersed in the liquid medium.
- Remove the ventilation needle and throw it into the sharps collector before transporting the
   T-I to the reference laboratory.

### 3.10.3 Transferring CSF into a cryotube for PCR

Meningitis, leptospirosis, and arboviroses

### **Equipment**

- CRYOTUBE, 2.0 mL, conical, ext. thread, sterile DNA/RNAse free [ELABTUMC20EP]
- PIPETTE, TRANSFER, graduated, plastic, sterile, s.u. [ELABPIPT1S-]
- RACK, PK, 6x4 microtubes, autoclavable [ELABTUMR24PK]
- STORAGE BOX, PP, 9x9 microtubes 1-2 mL, autoclavable [ELABTUMB81PP]
- Label the sterile cryotube with the patient's unique identification number and the collection date and location.
- After collecting CSF for inoculation into T-I, use a sterile transfer pipette/automatic pipette
  with a filter tip to remove the amount of CSF needed, depending on the disease in question,
  and transfer it to the cryotube.
- Reclose the cryotube by screwing the cap all the way down .

# **Chapter 4: Hygiene and safety**

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## 4.1 Standard precautions

Standard precautions are measures used in healthcare facilities to prevent the spread of microorganisms, not only between caregivers, patients, patient carers, and the community, but also between the patient and the environment.

While work clothes, handwashing, and gloves are required for handling any body substances, additional personal protective equipment should be considered depending on the environment, the suspected pathogen type, and the potential for splashing or spraying.

More details can be found in IPC-Pillar 3 Transmission-Based Precautions Guideline, MSF intersectional, 02/2020.

## 4.2 Personal protective equipment

Pathogens can be spread in a variety of ways: by inhalation, direct contact, ingestion, or inoculation.

Personal protective equipment (PPE) is used to prevent contamination of not just the caregiver, but the patient and the environment as well. The PPE that is used will depend on the suspected pathogen.

- Gloves: all rings and bracelets must be removed. Hands should be clean and dry before putting on gloves. Nails should be short to prevent damage to gloves.
  - Gloves are mandatory whenever caring for patients or handling samples, soiled linens, or medical waste. Some situations require double-gloving when collecting samples during surgery, in particular.
  - Hands must be washed or disinfected after removing gloves.
- Work clothing: an integral part of good personal hygiene. It helps reduce the risk of infection due to the transmission of microorganisms in the environment, protects the caregiver from being spattered or sprayed with body fluids or reagents, and protects the patient. Work clothing should be changed every day and whenever soiled. To ensure the quality of the cleaning and prevent contamination of the family environment, work clothes should not be cleaned at home
- Eye protection: goggles or face shields are used to protect the caregiver from droplets when there is a risk of spatter from blood or other body fluids or poisonous liquids and/or irritants.
   They are reusable, but cleaning/disinfection is essential.
- Face protection: surgical mask or respirator.
  - Surgical masks (type IIR) are worn by medical staff and contagious patients. They help
    protect those around the wearer, and the environment, from droplet-borne pathogens.
    They do not protect the wearer from airborne infections, but against infections transmitted
    by droplets. They should be replaced every 3 hours or whenever they become soiled, wet,
    or damaged.
  - With the COVID-19 pandemic, the WHO recommends that health workers wear a surgical
    mask at all times, and a respirator (N95/FFP2) during aerosol-generating procedures.
    Universal masking (by staff, patients, visitors, service providers, and others) in healthcare
    facilities and by hospitalised patients when it is impossible to maintain at least one metre
    of physical distance, or when patients are outside healthcare facilities, is recommended
    in areas with community spread.

Respirators (FFP2 or N95) help protect the wearer from airborne or droplet-transmitted respiratory diseases. They can be used for varying lengths of time, depending on the pathogen. Check with an IPC advisor for more details. They should be replaced when wet or damaged.

**Table 4.1** - TType of PPE required by transmission type (IPC-Pillar 3 Transmission-Based Precautions Guideline, MSF intersectional, 02/2020).

Equipment			Precauti	on type	
		Contact	Contact + droplet	Airborne	Airborne + contact
	Non-sterile gloves	Х	Х		X
	Apron/gown	Х	Х		Х
PPE	Surgical mask		Х		
	Respirator (FFP2/N95)		If VHF	Х	Х
	Eye protection		Х		

Special equipment is required when dealing with viral haemorrhagic fevers (ask the laboratory advisor, if needed).

- APRON PROTECTION, PVC, reusable [ELINAPRP1P-]
- APRON SURGICAL, rubber [ELINAPRS1R-]
- COAT, MEDICAL, white, short sleeve [ELINCOAW1+++]
- TROUSERS, SURGICAL, woven [ELINTROS1W+++]
- TUNIC, SURGICAL, woven [ELINTUNS1W+++]
- GLASSES, PROTECTIVE, plastic [EMEQGLAS1P-]
- GLOVE, EXAMINATION, latex, s.u. non sterile [SMSUGLOE1+++]
- GLOVES, SURGICAL, latex, s.u., sterile, pair, 7.5 [SMSUGLOS75-]
- COVERALL, hooded, s.u. (Tychem 2000 C CHA5 ) [SPPECVPHT+++]
- GLOVE, EXAMINATION, nitrile., extended cuff, s.u., non ster. [SPPEGLENE+++]
- GLOVES protective, nitrile, size 7, reusable, pair [PSAFGLOVN07]
- GLOVES protective, nitrile, size 8, reusable, pair [PSAFGLOVN08]
- GOGGLES PANORAMIC, regular nose, no ventilation [SPPEGOGPRN1]
- (goggles) ANTIMIST SPRAY, 500 mL [SPPEGOGPS5-]
- GOGGLES PANORAMIC, regular nose, indirect ventilation [SPPEGOGPRV1-]
- (goggles indirect ventilation, Ultravision) PANORAMIC LENS [SPPEGOGPU101]
- GOGGLES PANORAMIC, wide nose, indirect ventilation [SPPEGOGPWV1]
- (goggles flat nose Ultravision) PANORAMIC LENS [SPPEGOGPU201]
- LABORATORY GOWN, nonwoven, disposable [SPPEGOWNS1+++]
- HOOD, non-woven, integrated mask, VHF, s.u [SPPEHOOD2--]

## 4.3 Respiratory hygiene

A surgical mask must be worn (by the sick person) when a caregiver or patient has a cough of infectious origin.

Medical personnel should wear a surgical mask when the patient is suspected of having a droplet-transmitted disease (pertussis, rubella, etc.). For tuberculosis, measles, plague, or viral haemorrhagic fevers, care staff and visitors should wear a respirator.

- FACE SPLASH SHIELD, antifog, 19x33 cm + headband, reus., PPE [ELINFSSHR01]
- RESPIRATOR FFP2/N95 + IIR, unvalved, duckbill L [ELINMASP02L]
- RESPIRATOR FFP2/N95 + IIR, unvalved, 3-panel design, M [ELINMASP03M]
- MASK, SURGICAL, IIR type, s.u. [ELINMASS3--]

**Table 4.2** - Type of respiratory protection for staff and patient carers by suspected pathogen (IPC-Pillar 3 Transmission-Based Precautions Guideline, MSF intersectional, 02/2020 and COVID-19 intersection group IPC contact group – 07/2020)

Disease	Mode of transmission	Respiratory protection
VHF	Droplets and contact	Respirator (FFP2/N95)
Measles	Airborne and contact	Respirator (FFP2/N95)
Rubella (congenital)	Droplets and contact	Surgical mask
COVID-19	Airborne, droplets, contact, and aerosol	Surgical mask
Pertussis	Droplets and contact	Surgical mask
Respiratory diphtheria	Droplets and contact	Surgical mask
Meningitis	Droplets and contact	Surgical mask
Plague (pneumonic)	Airborne, droplets, and contact	Respirator (FFP2/N95)

## 4.4 Staff vaccination

In accordance with general MSF recommendations, MSF staff members are vaccinated against tetanus and hepatitis B. To the extent possible, we recommend vaccinating all personnel, including Ministry of Health staff working in MSF-supported projects — if necessary, by supplying the vaccines

For outbreaks of vaccine-preventable diseases and in specific situations where the staff that collects and/or handles samples is at risk of becoming infected (dengue, yellow fever, Japanese encephalitis, Covid-19, Ebola, polio, measles, rubella, cholera, typhoid fever, pertussis, diphtheria, and meningitis), the medical coordination team should check the current MSF recommendations on personnel protection in order to determine the best prevention or immunisation strategy. Vaccination of staff members, and even their families, should be facilitated – and perhaps organised – by MSF, if possible.

## 4.5 Asepsis, antisepsis, and disinfection

### 4.5.1 Asepsis

Using aseptic technique means working without bringing microorganisms into the work zone. This consists of using the means available (masks, gloves, sterile equipment, etc.) for performing the intended task.

#### 4.5.2 Antisepsis

Antisepsis consists of momentarily removing microorganisms from the skin using an antiseptic.

- CHLORHEXIDINE 2%, 70% isopropyl alcohol, SWAB/WIPE [DEXTCHLHA2W], 1st choice
- CHLORHEXIDINE 2%, 70% isopropyl alcohol, col. sol., 250 mL,bot.[DEXTCHLHA2S2C], 2<sup>nd</sup> choice
- POLYVIDONE IODINE 10%, solution (100 mL, 200 mL, fl. dropper bottle or 500 mL)
   [DEXTIODP1S-], 3<sup>rd</sup> choice
- CHLORHEXIDINE digluconate 2%, aqueous solution, 100 mL, bot. [DEXTCHLH2AS], for noenatology

#### 4.5.3 Disinfection

Disinfection is the removal of microorganisms from a work surface or an object. Disinfection is the operation that momentarily results in the removal or killing of microorganisms and/or inactivation of unwanted viruses on contaminated inert surfaces in accordance with set objectives.

## 4.6 Waste management

Proper management of medical waste helps ensure the safety of people and prevents the dissemination of infectious agents in the environment.

#### 4.6.1 Types of waste

Dans tous les cas, le matériel et les restes d'échantillons doivent être manipulés avec précaution car considérés comme infectieux.

- Sharps (needles, scalpel blades, pipette tips, etc.)
  - Do not recap needles.
  - Do not detach needles manually.
  - Discard needles or other sharps immediately after use, without handling, into an appropriate sharps collector located as close as possible to where the procedure is performed; check the maximum fill line regularly.
  - Never force sharps waste into the waste collector.
  - Never walk around with soiled needles or other sharps.
  - Never put soiled needles or other sharps down on the work surface.
- Soiled single-use equipment (transfer pipettes, tongue depressors, urine sample cups, etc.)
   and unused biological material.

#### 4.6.2 Types of disposal

There are several possible types of disposal, and these methods can be combined to optimise waste disposal.

The choice of disposal method depends on the suspected pathogen and the nature of the waste. The headquarters logistics and medical advisors should be involved in that choice.

#### **Decontamination**

Disinfectants (chlorine, Surfanios®/Hexanios®, etc.) can be used if they are proven to destroy the pathogenic microorganisms.

In some cases, autoclaving in steam should be considered, if available in the field.

- CHLORINE NaDCC, 1 kg, granules, pot [CWATDISING1]
- (NaDCC) MEASURE SPOON, 20 mL, ±15 g [CWATDISIHMS]
- CHLORINE, 1 g (NaDCC / dichloroisocyan. sodium 1.67 g), tab. [SDISNADC1T-]
- DETERGENT/DISINFECTANT for surfaces [SDISSUQA+++]

#### Incineration

A sufficiently powerful incinerator is needed to properly incinerate contaminated materials and samples. For some types of waste (biological and chemical agents), a medium temperature incinerator will suffice (850 °C post-combustion); otherwise, a high temperature incinerator is required.

# **Chapter 5: Test request forms**

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# 5.1 Arboviroses: dengue, yellow fever, West Nile fever, Japanese encephalitis, Zika and Chikungunya

REQUESTER	PATIENT
Country: Region: Town: Treatment centre: Test ordered by Dr. Project email:	Unique identification no.: Age: Sex: Occupation/activities: Place of residence:
History of the illness:	
Date of symptom onset:  Previous vaccinations with the number	Visit/admission date: of doses, dates, and types of arbovirus vaccines:
Source of information:  Card	History
Signs and Clinical examination °C fever for the past days Joint pain Meningeal signs Hepatic signs	<ul> <li>☐ Headache, muscle pain</li> <li>☐ Rash</li> <li>☐ Other:</li> <li>☐ Haemorrhagic signs</li> <li>☐ Renal signs</li> <li>☐ Pregnant</li> </ul>
_	egative; IgM:  Positive  Négatif; IgG: Positive  Negative  Positive  Negative Date:
Treatment Start date: Which/d	lose:
Clinical course (with dates of improve	ement, exacerbation, or death):
Suspected disease:	
Sample: date collected: Type of sample:  Serum Plasma D  Test requested:	ried blood spots (DBS)

## **5.2 COVID-19**

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Previous vaccinations with the number of dose	es, dates, and types of vaccines:
Source of information:   Card  B	History
Signs and Clinical examination	
At least one of the following major acute-on	set symptoms with no other obvious cause:
☐ Cough ☐ Dyspnoea	
☐ Dysgeusia ☐ Anosmia	
Or at least two of the following minor sympt	coms with no other obvious cause:
☐ Muscle pain ☐ Fatigue	
Headache Anorexia	
Sudden fall C fev	ver for the past days
	symptoms (COPD, asthma, chronic cough) with no other
Laboratory tests	
Rapid antigen test:	Date :
Rapid antibody test:	
Risk factors, exposure	
☐ Contact with confirmed case ☐ F☐ Other	Returned from an area with active transmission
Treatment	
Start date: Which:	
Clinical course with dates of improvement, e	exacerbation, or death:
Context: Outbreak investigation	☐ Monitoring during outbreak ☐ End of outbreak
Sample: date collected:	
Type of sample:	
	sopharyngeal swab
Test requested:	

# **5.3 Viral haemorrhagic fevers**

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email: ´	Place of residence:
History of the illness:	
Date of symptom onset:	
Previous vaccinations with the number of doses, dates,	and types of vaccines:
Source of information:	
Signs and Clinical examination	
At least one of the following major acute-onset symp	otoms with no other obvious cause:
°C fever for the past days	☐ Diarrhoea
	oint pain
☐ Melena/Hematemesis ☐ Abdomina	al pain 🗌 Jaundice
Petechiae Headache	!
Bleeding at injection sites Other hae	emorrhagic signs:
Pregnant Neurologi	cal signs Abdomen, liver, spleen
	s signs Other:
Laboratory tests	
☐ Malaria RDT/thick smear: Done ☐ Positive	Negative Date:
Urine - results :	
Other :	Date :
Risk factors, exposure	_
<ul><li>Contact with suspected cases or with animals or n</li><li>Stay in forests/caves with monkeys/bats</li></ul>	meat Stay in an outbreak area
Treatment	
Start date: Which:	
Clinical course (with dates of improvement, exacerba	ation, or death):
Suspected disease:	
Sample: date collected:	
Type of sample:	
Whole blood Buccal swab	
Test requested:	
ICSL I CUUCSICU.	

## **5.4 Hepatitis E**

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
	Visit/admission date:
Previous vaccinations with the number o	f doses, dates, and types of vaccines:
Source of information:	History
Signs and Clinical examination	
°C fever for the past days	☐ Jaundice ☐ Vomiting
Abdominal pain	Other:
☐ Pregnant	☐ Cutaneous signs ☐ Neurological signs
Abdomen, liver, spleen	Pulmonary signss
Laboratory tests	
ALT:	Date:
Other:	
Risk factors, exposure	
Contact with a confirmed case	
Treatment	
Start date: Which: _	
Clinical course (with dates of improven	nent, exacerbation, or death):
Context: Outbreak investigatio	n Monitoring during outbreak End of outbreak
Sample: date collected:	_
Type of sample:	
	ried blood spot (DBS)
Test requested:	

# **5.5 Poliomyelitis**

REQUESTER	PATIENT
Country: Region: Town: Treatment centre: Test ordered by Dr. Project email:	Unique identification no.: Age: Sex: Occupation/activities: Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Previous polio vaccinations with the number of doses, d	ates, and types of vaccines:
Source of information:   Card History	
Signs and Clinical examination	
°C fever for the past days Paralysis; site:	
☐ Gradual onset ☐ Rapid onset ☐ Sensitivity	☐ Urinary incontinence☐ Deep tendon reflexes
Laboratory tests	
Treatment Start date: Which:	
Clinical course (with dates of improvement, exacerba	tion, or death):
Sample: Date collected: First s Type of sample: Stool Test requested:	ample

## **5.6 PoxVirus**

REQUESTER	PATIENT	
Country: Region: Town: Treatment centre: Test ordered by Dr. Project email:	Age: Sex: Occupati	dentification no.: ion/activities: residence:
History of the illness:		
Date of symptom onset:	Visit/adm	nission date:
Signs and Clinical examination		
°C fever for the past d	ays Other:	
☐ Cutaneous signs ; location: ☐ Vesicles ☐ Pustules ☐ Scabs ☐ Lymphadenopathy ; location:  Laboratory tests	Pressure ulcer Satellite lymphangitis	
Treatment Start date: Wh	iich:	
Clinical course (with dates of impl	ovement, exacerbation, or d	eath):
	Vesicle fluid	Scabs
Test requested:		

## 5.7 Measles and rubella

REQUESTER	PATIENT	
Country: Region:	Unique identificat	ion no.:
Town:	Age:	
Treatment centre:	Sex:	
Test ordered by Dr.	Occupation/activi	
Project email:	Place of residence	•
History of the illness:		
Date of symptom onset:	Visit/admission dat	re:
Previous measles/rubella vaccinations	with the number of doses, dates,	and types of vaccines:
Source of information:   Card  Other vaccinations, with dates:	•	
Other vaccinations, with dates:		
Signs and Clinical examination		
At least one of the following major sy	• •	thout other evident cause:
°C fever	Cough	
Rhinorrhoea	☐ Conjunctivitis	
Cutaneous and mucous signs		
☐ Koplik spots	☐ Rash ☐ Des	squamation
Signs of secondary lung infection	☐ Diarrhoea ☐ Sig	ns of dehydration
Laboratory tests		
Treatment		
Start date: Which:		
Clinical course (with dates of improve	ment, exacerbation, or death): _	
Suspected disease:		
•		
Contexte : Outbreak investigation		
Context: date collected:		
Type of sample:		
Serum	Dried blood spot (DBS)	
Test requested:		

## 5.8 Cholera

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
Date of symptom onset:	
Previous vaccinations with the number of doses, da	tes, and types of vaccines:
Source of information:   Card History	
Signs and clinical examination	
Diarrhoea: frequency:	colour:
☐ Vomiting: frequency:	colour:
☐ Impaired consciousness	
Coma	
Signs of dehydration: moderate severe	
Signs of shock	
Other:	
Laboratory tests	
RDT done :	☐ Negative RDT Date:
Treatment	
Start date: Which:	
Clinical course (with dates of improvement, exacers	pation, or death):
Context: date collected:	
Type of sample:	
☐ Stool on Cary Blair® ☐ Stool on filter pa	aper Rectal swab in Cary-Blair®
Test requested:	

# **5.9 Typhoid fever**

REQUESTER	PATIENT
Country: Region: Town: Treatment centre: Test ordered by Dr. Project email:	Unique identification no.: Age: Sex: Occupation/activities: Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Previous typhoid vaccinations with the number	of doses, dates, and types of vaccines:
Source of information:   Card Histor	γ
Signs and Clinical examination °C fever for the past days Abdominal pain Asthenia, Anorexia Cutaneous signs: rose spots Splenomegaly Acute abdomen  Laboratory tests Malaria RDT/Thick smear: Done Po Complete Blood Count: WBC differential:	☐ Headache ☐ Epistaxis   ☐ Diarrhoea/constipation ☐ Prostration, confusion   ☐ Neurological signs ☐ Pulse:   ☐ Other: ☐ Other:    Sitive ☐ Negative ☐ Date:  Date:
Treatment Start date: Which:	
Clinical course (with dates of improvement, exa	acerbation, or death):
Suspected disease:	
Sample: date and time collected:  Date antibiotics started:  Type of sample: blood culture	
Test requested:	

# **5.10 Shigellosis**

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Signs and Clinical examination	
C fever for the past days	Abdominal pain
History of blood in the stools	Diarrhoea: frequency
_ ,	
Weight loss, malnutrition	Other:
☐ Signs of dehydration: ☐ moderate ☐ severe	e
Impaired consciousness, convulsions, coma	☐ Blood in the stools (macroscopic)
Other:	
Laboratory tests	
Stool microscopy: results:	Date:
	Butc
Risk factors, exposure	
Contact with people who had bloody diarrhoe	a
contact with people who had bloody didiffice	u
Treatment	
Start date: Which:	
Clinical course (with dates of improvement, exace	erbation, or death):
Suspected disease:	
Juspecteu disease.	
Sample: date collected:	
Type of sample:	
Faecal swab in Amies w/Charcoal	Rectal swab in Amies w/Charcoal
☐ Faecal swab in Cary Blair® ☐	Rectal swab in Cary Blair®
Test requested:	•
rest requested.	

# **5.11 Whooping cough (pertussis)**

PATIENT
Unique identification no.: Age: Sex: Occupation/activities: Place of residence:
Visit/admission date:es, and types of vaccines:
ooping" cough nea/cyanosis ht loss
ation, or death):
al swab

# **5.12 Diphtheria**

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Previous vaccinations with the numb	per of doses, dates, and types of vaccines:
Source of information.	
Source of information:   Card	·
Other vaccinations, with dates:	
Signs and Clinical examination	
C fever for the past days	S
Pseudomembranous tonsillitis	Lymphadenopathy
Laryngitis	Cervical oedema
Skin lesions	
Skill lesions	Other:
Laboratory tests	
Treatment	
Start date: Which	
Date antibiotics started:	Which:
Date antitoxin started:	Which:
Clinical course (with dates of improv	rement, exacerbation, or death):
Context: Outbreak investigation	on
Sample: date collected:	
	Date antitoxin started:
Type of sample:	
	aso-pharyngeal swab
Test requested:	

# **5.13 Leptospirosis**

REQUESTER	PATIE	NT		
Country:				
Region:	Uniqu	e identifica	tion no.:	
Town:	Age:			
Treatment centre:	Sex:			
Test ordered by Dr.		ation/activ		
Project email:	Place	of residence	e:	
History of the illness:				
				_
Date of symptom onset:			te:	-
Previous vaccinations (hepatitis, YF, etc				-
				- -
Signs and Clinical examination				
°C fever for the past days	Headache		☐ Jaundice	
Muscle/joint pain	Abdominal pain		☐ Diarrhoea/vomiting	
Petechiae	•			_
Neurological signs	☐ Conjunctival haer	morrhage		
Pulmonary signs	Other:			_
Laboratory tests				
Malaria RDT/Thick smear: Dor	ne Dositive D	Negative	Date :	
Complete Blood Count: WBC differe	ential:			
Urine: Results:				
Other:			Date :	_
Risk factors, exposure				
Contact with contaminated water (		)		
Contact with suspected cases				
Treatment				
Start date: Which:				_
				_
Clinical course (with dates of improver	ment, exacerbation, or	r death): _		_
				-
Sample: date collected:	-			
Type of sample:	_			
Whole blood (EDTA)	um CS	F	☐ Urine	
Test requested:				

# **5.14 Meningococcal meningitis**

REQUESTER	PATIENT	
Country:		
Region:	Unique identification no	.:
Town:	Age:	
Treatment centre:	Sex:	
Test ordered by Dr.	Occupation/activities:	
Project email:	Place of residence:	
History of the illness:		
Date of symptom onset:	Visit/admission date:	
	of doses, dates, and types of vaccines:	
Source of information:   Card	History	
Drugs taken since onset of symptoms:_		
Signs and Clinical examination		
°C fever for the past days	Headache	
□ Vomiting	Photophobia	
Stiff neck	☐ Brudzinski's or Kernig's sign	
☐ Bulging fontanelle	Seizures	Coma
	Seizures	Coma
Laboratory tests		
Malaria RDT/Thick smear: Done	e 🗌 Positive 🗌 Negative	Date :
Lumbar puncture: - results:		Date :
CSF colour:		
Cell count:		
Gram stain:		
Pastorex®:		
Treatment		
Start date: Which:		
Clinical course (with dates of improven	nent, exacerbation, or death):	
Context: Outbreak investigation	☐ Monitoring during outbreak	End of outbreak
Sample: Date collected:		
	Dose :	
Type of sample:	······································	
☐ CSF in Trans-Isolate ☐ CSF		
Test requested:		

## **5.15** Plague

REQUESTER	PATIENT
Country:	
Region:	Unique identification no.:
Town:	Age:
Treatment centre:	Sex:
Test ordered by Dr.	Occupation/activities:
Project email:	Place of residence:
History of the illness:	
Date of symptom onset:	Visit/admission date:
Signes et Examen clinique	
°C fever for the past days	☐ Chills, muscle pain
Painful lymph node	Headache
Coughing fits	Haemoptysis
Respiratory distress	
Bubo	Pulmonary signs
Septicaemic signs	☐ Meningeal signs
Laboratory tests	
Risk factors, exposure	
Presence of rats	Presence of fleas
	Fresence of fleas
Treatment	
Start date: Which: _	
Clinical course (with dates of improvem	ent, exacerbation, or death):
Sample: Date collected:	
Date antibiotics started:	
Type of sample:	
Swab of bubo pus (in Cary-Blair®or d	ry) Sputum
Sputum swab (in Cary-Blair® or dry)	
Test requested:	

# **Chapter 6: Sample transport**

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## **6.1 Legal provisions**

Biological samples are considered "dangerous substances" whose transport is subject to strict regulation based on the United Nations "Recommendations on the Transport of Dangerous Goods".<sup>a</sup>, <sup>1</sup>

The International Air Transport Association (IATA) has incorporated these recommendations into its "Dangerous Goods Regulations," to which many airlines are subject, including express transporters like DHL. These regulations are updated annually.

International air transport regulations (IATA) are the most restrictive.

Whatever the type of transport – national or international, road, rail, maritime, or air – all MSF shipments of potentially infectious samples must comply with those regulations.

According to the "Recommendations on the Transport of Dangerous Goods", all biological samples fall into the "6.2-Infectious Substances" risk class.

<sup>&</sup>lt;sup>a</sup> UN Library, 2019. Recommendations on the Transport of Dangerous Goods: Model Regulations. https://doi.org/10.18356/7c03b465-en

## **6.2 Sample classification**

Infectious substances are divided into three categories according to risk:

Infectious substance, Category A Classification UN2814	An infectious substance that is transported in a form that, when exposure to it occurs, is capable of causing permanent disability, lifethreatening or fatal disease in otherwise healthy humans or animals
Biological substance, Category B Classification UN3373	A biological substance that does not meet the criteria for Category A, but which contains pathogens capable of infecting humans or animals, with no risk of death or permanent disability.  Samples that contain pathogens not classified as UN2814 or not included in the exemptions (slides or dried blood spots (DBS)) should be considered as UN3373.
Exemptions	Some biological products do not fit into Category A or B for a variety of reasons and hence are exempt; the IATA regulations do not apply.

Information regarding the danger category for each of the pathogens described in the guidelines can be found in Chapter 2. A table of all Category A infectious substances – UN2814 can be found in the MSF catalogue, volumes 5, 6 and 8 (see Section 6.6.1).

A declaration is also required when using dry ice as a cooling agent, because it is a dangerous substance. The following must be added to the packages in question: UN1845 "Carbon dioxide, solid" (+ specific hazard label). The following must also appear on the Air Waybill (AWB): UN1845 "Carbon dioxide, solid", the number of packages, and the net quantity of dry ice added per package. This must also appear on the Dangerous Goods Declaration, if it accompanies UN2814 infectious substances.

Every type of international transport has specific documents and conditions regarding:

- The packing instruction
- Marking and labelling
- Transport mode
- Administrative documentation

#### It is therefore necessary to:

- Assign the dangerous substance to be transported to one of the three categories (UN2814, UN3373, or exemptions). It is up to the medical staff to determine which categories the samples in question belong to.
- Know the recommended transport temperature: ambient temperature or cold chain (positive or negative).
- If there is any doubt or question, consult the laboratory advisor.

### 6.2.1 Special case of dried blood spot (DBS) shipments

According to the IATA, DBS shipments are exempt.<sup>2</sup>

The completely dry DBS should be placed in its own bag with desiccant, and the bag placed in an envelope and shipped by a rapid transporter with shipment tracking (e.g., DHL, UPS, or FedEx).

DBS samples shipped via transporter must meet the following criteria:

- Triple packaged, with two waterproof inner packagings (individual bags and then a zip-lock bag).
- A third (outer) packaging (envelope or cardboard box) measuring at least 10 x 10 cm.
- "Exempt Human Specimen" appears on the package (outer packaging) and on the Air Waybill.

The transporter may ask for documentation proving that the samples are exempt.<sup>3</sup> Check with the laboratory advisor.

## 6.3 Biological substance, Category B - UN3373

### 6.3.1 Transport materials, transport temperature, and packing instruction

Regardless of transport temperature, the packaging must consist of the following three components:

- A primary receptacle
- A secondary\* packaging, and
- A rigid outer packaging
- \* For liquid biological substances, enough absorbent material should be placed between the primary receptacle(s) and the secondary packaging to absorb the entire contents in case of leakage. Each primary sample should be individually wrapped so that there is no contact between samples.

The maximum volume of the primary receptacle is 1 litre, and the outer packaging should contain no more than 4 litres of dangerous liquid substances, e.g., blood or urine samples, or 4 kg of dangerous solid substances (e.g., swabs or Trans-Isolate). This weight or volume does not include ice packs or dry ice.

Note: depending on the quantity of dangerous substances to be shipped, check the maximum weight or volume for which the packaging was manufactured, which may be less than the quantities above. The manufacturer will provide the product characteristics.

- Ambient temperature transport: IATA packing instruction 650
   Two items available in the MSF catalogue:
  - BOX, triple packaging, biological substance UN3373 +pouch [STSSUN62DS-] (Figure 6.1)
  - BOX, triple packaging, biological substance UN3373+container [STSSUN62DS2]
  - + POUCH, polyethylene, for transport of samples, 20 x 30 cm [ELABPOUP203]
- Cold chain transport between +2 and +8 °C: IATA packing instruction 650
   Two items available in the MSF catalogue:
  - BOX ISOTHERMAL, triple pack., biological subst. UN3373 +pouch [STSSUN62DSI] (Figure 6.2)
  - BOITE ISOTHERME, triple emb, substance biologique UN3373 + récipient [STSSUN62DSI2]



Figure 6.1 - Shipping material UN3373
Ambient temperature



Figure 6.2 - Shipping material UN3373 Cold chain

The temperature is controlled by four ice packs that are pre-frozen and placed between the secondary and outer packaging.

With four ice packs, the insulated box should keep the samples between +2 °C and +8 °C for 48 hours at an ambient temperature of +28 °C.

Additional materials available:

- CONTAINER, PROTECTION, transport of sample, plastic, Ø 30 mm [STSSCONP030P]
- − CONTAINER, PROTECTION, transport of sample, plastic, Ø 44 mm [STSSCONP044P]
- − CONTAINER, PROTECTION, transport of sample, plastic, Ø 130mm [STSSCONP130P]

### 6.3.2 Marking and labelling

The regulations on marking and labelling for ambient temperature transport and cold chain (+2 °C to +8 °C) transport are the same. Additional information and a hazard label are required on outer packaging containing dry ice (see Section 6.2).

The outer packaging should display the following information:

- The following pictograph, measuring at least 50 mm a side, with the text "Biological substance, Category B" in letters at least 6 mm high adjacent to the pictograph.



Figure 6.3 - Pictograph UN3373

- The name and physical address of the recipient must be entered in the spaces provided (no post office boxes but any details that might facilitate direct delivery).
- The name and physical address of the sender should be entered in the spaces provided.
- The name and telephone number of someone in the field who can be contacted 24 hours a day should be entered in the spaces provided.
- Shipments to the Institut Pasteur laboratory in France must bear the Institute's own shipping label (voir Section 6.6.5).

#### **6.3.3 Transport modes**

DHL: biological substances, Category B - UN3373 can be shipped via DHL or other transporters or airlines.

For cold chain shipments, the transporter must be informed so that the package is placed in a refrigerated cargo container, if available.

#### Notes:

- DHL can transport refrigerated packages but does not maintain the cold chain (i.e., it does
  not replenish ice packs or add dry ice for cold chain shipments). The sender is responsible
  for ensuring that the cold chain system used (ice packs or dry ice) is sufficient for the entire
  duration of shipment.
- Some transporters/forwarding agents do monitor the cold chain, replenish ice packs, or add dry ice (World Courrier, for example). It is the sender's responsibility to enquire about the options ahead of time.

#### **6.3.4** Administrative documents

These are the responsibility of the supply and logistics departments.

Shipments of Biological Substances, Category B – UN3373 do not require dangerous goods declarations (DGD), even when shipped with dry ice.

- A donation certificate with the recipient's and sender's addresses, the exact name of the suspected pathogen and the quantities being shipped (completed by the supply department (see Section 6.6.3).
- A "custom invoice" specifying the monetary value of the product (prepared by the supply or logistics department) with the recipient's and sender's addresses, the number of packages, detailed contents, and weight.
- A Material Transfer Agreement issued by the Ministry of Health or other competent authority in the country.
- For some countries, an **import permit** is required and should be furnished by the receiving laboratory.

A paper copy of these documents is given to the forwarding agent in the originating country. If there is a forwarding agent involved in the destination country as well, all the documents must be provided to him in electronic form (logistics/supply department responsibility).

An anonymised packing list (without patient's name) should be inserted between the secondary and tertiary packaging of the package in question.
 A copy of the packing list and the package's anticipated arrival date should be emailed to the receiving laboratory.

The **forwarding agent**: an essential service provider for all air shipments of infectious or biological substances (UN2814 and UN3373). If shipment with DHL, DHL takes the role of forwarding agent.

- Is commissioned by MSF.
- Is the intermediary between MSF and the airline at departure and between the airline and the receiving laboratory.
- When shipping via DHL, DHL manages the entire shipment ("door-to-door" service). If not shipping via DHL, a forwarding agent is needed at departure and a second forwarding agent may be needed to collect the package at arrival and facilitate its passage through customs.
- Completes the air waybill (AWB) (see Section 6.6.4):
  - The AWB should show "UN3373 Biological substance, category B" but not the name of the infectious agent. It should also include the number of packages.
  - An accredited DHL account number must be used for UN3373 shipments. Contact the supply centre, laboratory advisor, or mission to get that account number.

When shipping via DHL, you must also notify the headquarters transport manager and forward the DHL tracking numbers (Air Waybill) (MSF-OCBA, MSF-OCG and MSF-OCP: MSF-Log focal point in Bordeaux; MSF-OCA: the transport officer at the APU and the mission laboratory advisor; MSF-OCB: laboratory advisor).

Other conditions may be required for cargo shipments. Contact the supply/logistics advisor and notify the medical department.

#### 6.4 Infectious substance, Category A - UN2814

#### 6.4.1 Transport materials, transport temperature, and packing instruction

Regardless of transport temperature, the packaging must consist of the following three components:

- A watertight, leak-proof primary receptacle(s)
- A watertight, leak-proof secondary packaging\*
- A rigid outer packaging
- \* For liquid infectious substances, enough absorbent material should be placed between the primary receptacle(s) and the secondary packaging to absorb the entire contents in case of leakage. Each primary sample should be individually wrapped so that there is no contact between the sampless.

Maximum allowable volume per package:

- 50 mL or 50 g for passenger plane transport,
- 4 litres or 4 kg for cargo plane transport

This weight or volume does not include ice packs or dry ice.

#### Ambient temperature, IATA packing instruction 620 One item available in the MSF catalogue:

BOX, triple packaging, infectious substance UN2814 [STSSUN62IS-]]

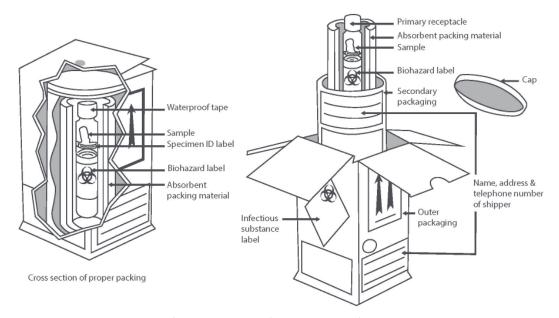


Figure 6.4 - Shipping material UN2814 - Ambient temperature



Figure 6.5 - Triple packaging box UN2814 - Ambient temperature

#### Cold chain transport between +2 and + 8 °C, IATA packing instruction 620

The temperature is controlled by four ice packs that are pre-frozen and placed between the secondary and outer packaging.

One item available in the MSF catalogue:

BOX ISOTHERMAL, triple pack., infectious substance UN2814 [STSSUN62ISI]

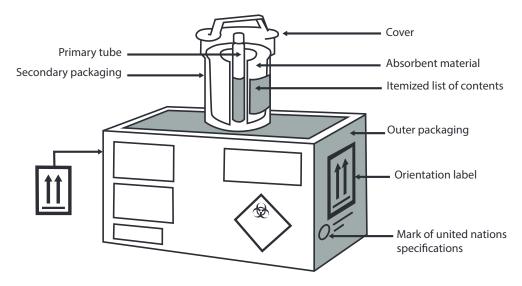


Figure 6.6 - Shipping material UN2814 - Cold chain



Figure 6.7 - Triple packaging box UN2814 - Cold chain

Insulated box that keeps the samples between +2 °C and +8 °C for 48 hours (at an ambient temperature of +28 °C).

#### Additional materials available:

- − CONTAINER, PROTECTION, transport of sample, plastic, Ø 30 mm [STSSCONP030P]
- − CONTAINER, PROTECTION, transport of sample, plastic, Ø 44 mm [STSSCONP044P]
- CONTAINER, PROTECTION, transport of sample, plastic, Ø 130mm [STSSCONP130P]
- POUCH, polyethylene, for transport of samples, 20 x 30 cm [ELABPOUP203]

#### 6.4.2 Marking and labelling

The regulations on marking and labelling for ambient temperature transport and cold chain (2 °C to +8 °C) transport are the same. Additional information and a hazard label are required on outer packaging containing dry ice (see Section 6.2).

The outer packaging should display the following information:

 The following pictograph (infectious substance symbol) (pre-printed on MSF materials) with UN2814 "Infectious substance, affecting humans".



Figure 6.8 - Pictograph infectious substance

- The name and physical address of the recipient must be entered in the spaces provided (no post office boxes but provide any details that might facilitate direct delivery).
- The name and physical address of the sender should be entered in the spaces provided.
- The name and telephone number of someone in the field who can be contacted 24 hours a day should be entered in the spaces provided.
- Shipments to the Institut Pasteur lab in France must bear the Institut's own shipping label (see Section 6.6.5).

#### 6.4.3 Transport modes

- Infectious substances, Category A UN2814 can be transported by an airline or possibly other transporters accredited by the IATA for UN2814 shipments (enquire locally). They cannot be transported by DHL.
- For cold chain shipments, the transporter must be informed so that the package is placed in a refrigerated cargo container, if available.
- Transporters can transport refrigerated packages, but not all of them maintain the cold chain (i.e., they do not replenish ice packs or add dry ice for cold chain shipments). The sender is responsible for ensuring that the cold chain system used (ice packs or dry ice) is sufficient for the entire duration of shipment.

#### 6.4.4 Administrative documents

These are the responsibility of the supply and logistics departments.

- A dangerous goods declaration (DGD): must include "Infectious substance, affecting humans (+ scientific name of the substance, if known, in parentheses)" or "Infectious substance, affecting humans (+ suspected Category A infectious substance, in brackets" (for example: "Infectious substance, affecting humans (Ebola virus)" or "Infectious substance, affecting human (suspected Category A infectious substance)") + the net quantity. (see example, Section 6.6.2).
  - *Note*: the DGD must be completed and signed by an IATA-accredited person. If this is not the case for MSF personnel or the local forwarding agent, contact the supply/logistics department and your laboratory advisor to get a list of accredited people within the departments.
- A donation certificate with the address of the sender and the recipient, the exact name of the suspected pathogen, and the quantities contained, completed by the supply department (see, Section 6.6.3).

- A custom invoice (with the monetary value of the product) prepared by the supply department or logistics coordinator, with the sender's and recipient's addresses, the number of packages, their contents, and their weight.
- A Material Transfer Agreement (MTA) issued by the Ministry of Health or other competent authority of the country.
- For some countries, an **import permit** is required in the recipient's country; this will have to be furnished by the receiving laboratory.

A paper copy of these documents is given to the forwarding agent in the originating country. If there is a forwarding agent involved in the destination country as well, all of the documents must be provided to him in electronic form (logistics/supply department responsibility).

An anonymised packing list (without patient's name) should be inserted between the secondary and tertiary packaging of the package in question.
 A copy of the packing list and the package's anticipated arrival date should be emailed to the receiving laboratory.

The forwarding agent: an essential service provider for all air shipments of infectious or biological substances (UN2814 or UN3373).

- Is commissioned by MSF.
- Is the intermediary between MSF and the airline and/or between the airline and the receiving laboratory.
- In some cases, a second forwarding agent may be needed to collect the package on arrival and facilitate its passage through customs.
- Completes and signs the DGD if he has been accredited by the IATA for less than two years.
- Completes the AWB (Air Waybill) (see Section 6.6.4):
  - The "handling information" box on the AWB must state "dangerous goods as per associated DGD".
  - The AWB should include "UN2814 Infectious substance, affecting humans", but not the name of the infectious agent.

Other conditions may be required for cargo shipments. Contact the supply/logistics advisor and notify the medical department.

#### 6.5 Cold chain preparation



The insulated boxes available in the catalogue will keep the temperature between +2 °C and +8 °C for 2 days at an ambient temperature of 28 °C. It has not been tested at higher ambient temperatures.

#### 6.5.1 Shipping at +2 °C to +8 °C with 0.6-litre ice packs

The procedure for samples that have to be sent at +2 °C to +8 °C using ice packs is as follows:

- Prepare an insulated triple-packaging box.
- Prepare the four ice packs:
  - Remove them from the freezer and leave them in a +20 °C location, protected from the sun.
  - After 15 minutes, check the amount of liquid in the ice packs. To ensure that the ice packs are at the right temperature, they should have 5 cm of water visible in the bottom when held vertically. When the ice packs are shaken, the sound of water can be heard.



If there is less than 5 cm of water or no water at all, the packs are too cold, and the **!** samples might freeze early in transport.

If there is more than 5 cm of water, the packs are too warm, and the temperature might get too high during transport.

- If the ice packs are not at the correct temperature after 15 minutes, leave them for another 15 minutes, protected from sunlight, and then check again.
- Once all four ice packs are at the correct temperature, place them in the insulated box.
- Put the samples, in their packaging, into the box.
- Close the insulated box.



Figure 6.9 - Ice pack

#### 6.6 Documentation

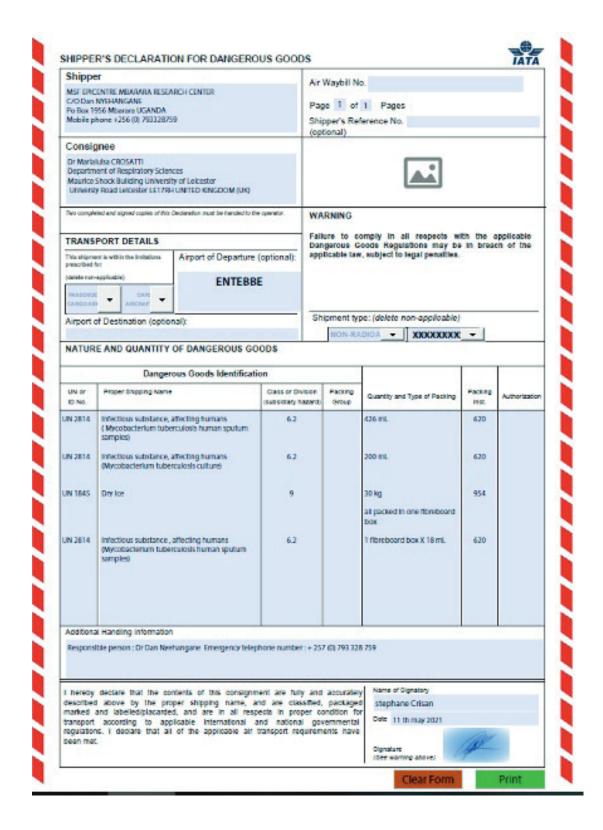
#### 6.6.1 IATA: Category A - UN2814

Indicative examples of infectious substances included in Category A in any form unless otherwise indicated

UN Number and Proper Shippin Name	Micro-organism	Form
UN2814	Bacillus anthracis	Cultures only
Infectious substance	Brucella abortus	Cultures only
affecting humans	Brucella melitensis	Cultures only
, and the second	Brucella suis	Cultures only
	Burkholderia mallei – Pseudomonas mallei – Glanders	Cultures only
	Burkholderia pseudomallei – Pseudomonas	Cultures only
	pseudomallei	Januar 25 2,
	Chlamydia psittaci – avian strains	Cultures only
	Clostridium botulinum	Cultures only
	Coccidioides immitis	Cultures only
	Coxiella burnetii	Cultures only
	Escherichia coli, verotoxigenic	Cultures only
	Francisella tularensis	Cultures only
	Hantavirus causing hemorrhagic fever with renal	Cultures only
	syndrome	
	Mycobacterium tuberculosis	Cultures only
	Poliovirus	Cultures only
	Rickettsia prowazekii	Cultures only
	Rickettsia rickettsii	Cultures only
		Cultures only
	Shigella dysenteriae type 1	
	Dengue virus	Cultures only
	Rift Valley fever virus	Cultures only
	Crimean-Congo haemorrhagic fever virus	
	Omsk haemorragic fever virus	
	Yellow fever virus	Cultures only
	Kyasanur Forest disease virus	
	Variola virus	
	Tick-borne encephalitis virus	Cultures only
	Eastern equine encephalitis virus	Cultures only
	Venezuelan equine encephalitis virus	Cultures only
	Japanese Encephalitis virus	Cultures only
	Russian spring-summer encephalitis virus	Cultures only
	Hepatitis B virus	Cultures only
	Human immunodeficiency virus	Cultures only
	Highly pathogenic avian influenza virus	Cultures only
	Marburg virus	
	West Nile virus	Cultures only
	Ebola virus	
	Flexal virus	
	Guanarito virus	
	Hantaan virus	
	Hendra virus	
	Herpes B virus	Cultures only
	Junin virus	,
	Lassa virus	
	Machupo virus	
	Monkeypox virus	
	Nipah virus	
	Rabies virus	Cultures only
	Sabia virus	23.124.25 3.117
	Yersinia pestis	Cultures only
	Teronina pestis	_ calcares offiny

Adapted from *Guidance on regulations for the transport of infectious substances 2021-2022*. Geneva: World Health Organization; 2021. Licence: CCBY-NC-SA3.0IGO

#### 6.6.2 Sample dangerous goods declaration (DGD)



#### **6.6.3 Sample donation certificate**



in Paris, January 30th 2016

#### CERTIFICAT DE DON CERTIFICADO DE DONACION FREE GIFT CERTIFICATE

Nous soussignés EPICENTRE certifie que les produits suivants :

We undersigned EPICENTRE certify that we make a donation without commercial value and Not DESIGNED FOR COMMERCIAL USE of the following products:

El abajo firmante por EPICENTRE certifica hacer une donacion sine alguna valor commercial de los productos siguentes :

#### MATERIEL POUR AIDE HUMANITAIRE HUMANITARIAN RELIEF GOODS MATERIAL PAR AYUDA HUMANITARIA

152 Stool samples (152 mL), UN3373 Biological Substance Category B
This is a free gift from Epicentre head quarter in France, to the Cincinnati Children's Hospital
Medical Center, USA, having no commercial value—used only for medical research

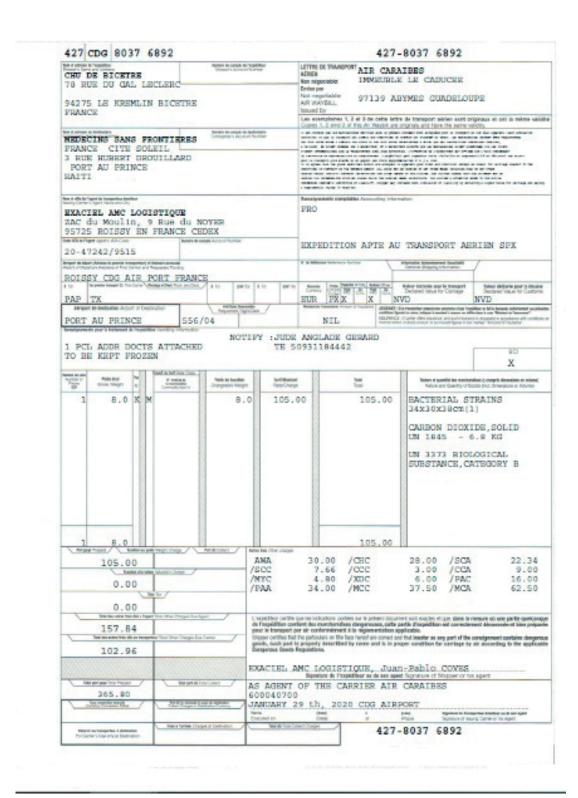
TOTAL VALUE: 10 USD taxes free

Country of final destination: United States of America

By Céline Langendorf, Laboratory coordinator for EPICENTRE

Signature:

#### 6.6.4 Sample Air Waybill (AWB)



#### 6.6.5 Institut Pasteur shipping label

Available at the following address:

https://www.pasteur.fr/fr/file/3157/download?token=Evu6jsP5

Quel que soit le mode de transport choisi, compléter et apposer une étiquet de tous les envois destinés aux Centres Nationaux de Référence d	•
EXPÉDITEUR (à compléter ou cachet du Laboratoire)	CONSERVER à :
NOM / RAISON SOCIALE	□ Température ambiante
Hopital / Laboratoire / Service	□ +4°C □ -20°C
ADRESSE	
CODE POSTAL VILLE VILLE PAYS	
TEL FAX	
DESTINATAIRE	
INSTITUT PASTEUR Centre National de Référence de A l'Attention de	
25 - 28 rue du Docteur Roux	
75 724 PARIS CEDEX 15	

Regardless of the mode of transport, fill out and affix a label as above to the outer packaging of all shipments destined for the Institut Pasteur's National Reference Centers.

#### Sender:

- Name
- Hospital/Laboratory/Unit
- Address
- Postal code
- City
- Country
- Phone number
- Fax number

#### Storage conditions: choose one

- Ambient temperature
- +4 °C
- -20 °C

#### Recipient:

- Institut Pasteur
- National reference laboratory of \_\_\_\_\_\_
- For the attention of \_
- Address: already added

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## Chapter 7: Registers

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7.3 Register to be kept in the CAPITAL	.161

#### 7.1 Introduction

There are two types of registers used for tracking samples.

The first should be completed and kept in the field (see Section 7.2). It contains information about the patient and details about the sample. The results are entered at the lab or by the medical officer. This register should be kept under lock and key (for confidentiality).

The second register should be kept in the capital (see Section 7.3). It too contains information about the sample, as well as details on the sample's shipment. It is completed by the medical coordinator (the sample portion) and by the logistics coordinator (the shipment info).

## 7.2 Register to be kept in the FIELD

Lab sample shipment register

Locality:

	Result									
Date	result	received								
Shipping method	Car	Plane	Other							
Sample category	UN2814	UN3373	Exemption							
Cold	CIIdilli	YES	NO							
Shipped		Capital	Lab							
	Date	naddills								
Previous lab tests	+ results									
Sample	adkı	Blood	CSF							
	Suspected	ulagilosis								
Provenance: (health centre)										
	Patient name									
	Sample ID									
	Date sample	מוברובת								

Note: confidential document with patient identifiers, do not share.

# 7.3 Register to be kept in the CAPITAL

Lab sample shipment register

Locality:

Year:

	Result										
4	result	received									
Shipping method	Car	Plane	Other								
Sample category	UN2814	UN3373	Exemption								
Cold	chain?	YES	NO								
	Shipped	Ē									
	Date	paddius									
Sample	type	Blood	CSF								
	Suspected	uldgillosis									
	Date sample	מופרופו									
	Provenance of Sample										
	Sample ID										
Date sample received in capital											

### **Chapter 8: List of laboratories and contacts**

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#### 8.1 Laboratories

#### Meningitis reference laboratory

Norwegian Institute of Public Health Attn: Dominique A. Caugant Geitmyrsveien 75 0403 OSLO Norway

Tel: +47 22 04 23 11 Fax: +47 22 04 25 18

Email: dominique.caugant@fhi.no

#### **Cholera reference laboratory (filter paper)**

Centre national de référence des Vibrions et du Choléra Unité de recherche et d'expertise des Bactéries pathogènes entériques Institut Pasteur 25-28 rue du Docteur Roux 75724 Paris Cedex 15 France

Telephone: +33.1 45 68 82 21 (Secretary's office),

+33 1 40 61 33 85

Fax: +33 1 45 68 88 37 Email: vibrions@pasteur.fr

Other reference laboratories (to be filled in by the medical coordination team)

Reference laboratory	Reference laboratory	
Name:		_
Contact person:		
Address:		
		_
Telephone:	Telephone:	
Email:		
Reference laboratory	Reference laboratory	
Name:	<del></del>	
Contact person:		
Address:		
Telephone:		
Email:	Email:	

Reference laboratory	Reference laboratory	
Name:	Name:	
Contact person:		
Address:		
Telephone:		
Email:	Email:	
Reference laboratory	Reference laboratory	
Name:	Name:	
Contact person:		
Address:		
Telephone:	Telephone:	
Email:		
Reference laboratory	Reference laboratory	
Name:		
Contact person:		
Address:		
Telephone:		
Email:	Email:	
Reference laboratory	Reference laboratory	
Name:	Name:	
Contact person:		
Address:	Address:	
Telephone:		
Email:	Email:	

#### 8.2 Contacts

#### Laboratory and/or WHO and/or CDC contacts made in writing this guide:

NRC [National Reference Centre] Arboviroses—IRBA - Marseilles

WHOCC Hamburg for VHFs

NRC Viral Haemorrhagic Fever - Pasteur Lyon

NRC Hepatitis - Toulouse

NRC Polio – Clermont-Ferrand

**CDC Poxvirus** 

NRC Poxvirus – IRBA - Marseilles

WHO Measles/Rubella

NRC Cholera – Pasteur Paris

NRC Salmonellosis – Pasteur Paris

WHO Salmonellosis – Pasteur Paris

**CDC Salmonellosis** 

NRC Shigellosis – Pasteur Paris

NRC Pertussis - Pasteur Paris

NRC Diphtheria – Pasteur Paris

NRC Leptospirosis – Pasteur Paris

WHOCC Meningitis – Norwegian Institute of Public Health Oslo

WHOCC and NRC Plague – Pasteur Paris

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