National Guideline for

SAMPLE COLLECTION AND TRANSPORTATION DURING ACUTE PUBLIC HEALTH EVENTS

2025

Government of Nepal
Ministry of Health and Population
Department of Health Services
Epidemiology and Disease Control Division

Government of Nepal
Ministry of Health and Population
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Preface

It is my great pleasure to present the *National Guideline on Sample Collection and Transportation during Acute Public Health Events*, developed by the Epidemiology and Disease Control Division in collaboration with the National Public Health Laboratory.

Timely and accurate diagnosis plays a pivotal role in the detection, confirmation, and effective response to public health emergencies. During recent outbreaks and emergencies, challenges related to the collection, packaging, and transportation of biological specimens were repeatedly identified as critical gaps in our response efforts. Addressing these challenges is key to improving our readiness and ability to respond swiftly to emerging threats.

This guideline provides a standardized and practical framework for health workers, laboratory staff, and response teams at all levels. It details step-by-step procedures for safe sample collection, labeling, packaging, storage, and transportation, while emphasizing adherence to biosafety and biosecurity standards. The aim is to strengthen the overall surveillance-to-laboratory linkage and ensure that collected specimens reach testing laboratories in optimal condition.

The guideline is intended to be used in conjunction with existing national protocols such as the Alert and Response Framework, Collaborative Surveillance, Rapid Response Team (RRT) and Emergency Medical Team (EMT) guidelines. Together, these tools will help to establish a more resilient and responsive health system capable of managing public health emergencies efficiently and effectively.

I would like to commend the technical team at the Epidemiology and Disease Control Division (EDCD), the National Public Health Laboratory (NPHL), World Health Organization (WHO), and all stakeholders for their tireless efforts in bringing this important document to fruition. I am confident that this guideline will serve as a valuable resource to support timely detection and response at all levels of the health system.

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Foreword

Timely and proper collection and transportation of clinical samples is one of the most critical components in public health emergency response. It enables accurate laboratory confirmation, early detection, and evidence-based interventions during outbreaks and other acute public health events. In the context of Nepal, where geographic, infrastructural, and logistical challenges can delay timely diagnosis, having a standardized, practical guideline is essential to ensuring a coordinated and effective response.

This *National Guideline on Sample Collection and Transportation during Acute Public Health Events* serves as a strategic tool for health workers, laboratories, emergency responders, and partner organizations across the country. It provides clear and actionable protocols that align with international best practices, while also reflecting the unique contextual realities of Nepal's geography, health infrastructure, and resource settings. The guideline emphasizes biosafety and biosecurity, packaging standards, and timely transportation to ensure safe and effective sample handling from the field to reference laboratories.

Importantly, the guideline promotes coordination between the Epidemiology and Disease Control Division (EDCD), Provincial Health Directorates, health offices and laboratories at all levels. It supports the efforts of Rapid Response Teams (RRTs) by establishing consistent procedures that enable swift and accurate detection of pathogens, ultimately facilitating prompt action to mitigate public health threats. By emphasizing coordination mechanisms across levels of government, the guideline promotes a holistic and inclusive approach to public health emergency preparedness and response.

I would like to acknowledge the efforts of the technical teams at EDCD and the National Public Health Laboratory (NPHL), as well as the valuable contributions of the World Health Organization and other partners. Their collaboration has been instrumental in developing this guideline.

The Epidemiology and Disease Control Division remains committed to strengthening national systems to respond to public health threats with greater efficiency and resilience. I encourage all stakeholders to operationalize this guideline across all levels of the health system to better safeguard the health and well-being of communities across Nepal.

Dr Chandra Bhal Jha
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Foreword

Laboratory services are the cornerstone of effective public health responses, providing the evidence required to detect, confirm, and monitor disease outbreaks. Timely and high-quality laboratory testing relies not only on technical capacity but also on the seamless coordination of sample collection, packaging, and transportation especially during public health emergencies.

The *National Guideline on Sample Collection and Transportation during Acute Public Health Events* represents a critical step toward strengthening Nepal's laboratory system and ensuring that samples are handled with the highest standards of biosafety, biosecurity, and diagnostic integrity. The document provides clear and practical guidance for rapid response teams, and laboratory personnel, involved at various stages of the sample collection and transportation from the field to the reference laboratories.

The National Public Health Laboratory (NPHL) serves as the apex and reference laboratory within the national laboratory structure, which includes laboratories across different levels of the health system. This structure spans across all seven provinces involving Province Public Health Laboratories (PPHLs), district hospital-based laboratories, local hospital-based laboratories, medical college laboratories, and private laboratories. This structured laboratory system ensures timely diagnostic services at multiple levels, supporting effective public health response and disease surveillance.

We recognize that the strength of our testing capacity is directly linked to the quality of the samples we receive. Poor handling or delays in transport can compromise diagnostic outcomes and affect the timeliness of public health interventions. This guideline addresses these challenges by establishing standardized procedures and the need for a networking and transport mechanism for sample transportation while promoting inter-sectoral collaboration.

I would like to express my gratitude to all the technical contributors and institutions, including EDCD, NPHL, and WHO, for their involvement in the development of this important document. Together, let us ensure its effective implementation to reinforce the foundation of Nepal's laboratory response system and enhance our preparedness for current and future health emergencies.

Dr Ranjan Raj Bhatta
Director
National Public Health Laboratory

World Health Organization

Nepal

Message from WHO Representative to Nepal

The capacity to rapidly detect, confirm and respond to public health emergencies constitutes the foundation of resilient health systems. Timely and accurate laboratory diagnosis is integral to this process, beginning with the safe and efficient collection, and transportation of clinical samples. This is not only a technical necessity but also a core obligation under the *International Health Regulations (IHR 2005)*, which call upon countries to develop and sustain the capacities to prevent, detect and respond to potential public health emergencies of international concern.

The *National Guideline on Sample Collection and Transportation during Acute Public Health Events* represents a significant step forward in operationalizing these global commitments at the national level. It provides a practical, context-sensitive framework to ensure that samples collected during emergencies are handled safely, transported efficiently, and tested reliably. The guideline is informed by best practices and lessons learned from past emergencies, while integrating critical elements of biosafety, biosecurity, equity, and coordination across all levels of the health system.

Given its broad applicability and relevance across sectors, this document strengthens existing policies and guidelines by promoting coherence and harmonization across different tiers of the laboratory system and public health initiatives. This coordinated approach will foster seamless integration among relevant stakeholders, facilitating a unified and effective response during public health emergencies.

This guideline is closely aligned with WHO's global strategy on health emergency preparedness and supports Nepal's progress toward IHR compliance and the broader goals of the Sustainable Development Agenda. By strengthening national capacities, Nepal is contributing not only to its own health system resilience but also to broader regional and global efforts toward universal health security.

The World Health Organization is proud to support the Government of Nepal in this important endeavor. We commend the leadership of the Ministry of Health and Population and the Department of Health Services, the technical contributions of the Epidemiology and Disease Control Division and the National Public Health Laboratory, and the dedication of everyone involved in this initiative.

Let this guideline serve not only as a tool for emergency response, but also as a testament to Nepal's commitment to building a safer and healthier future for all.

RSPandav

Dr Rajesh Sambhajirao Pandav
WHO Representative to Nepal
World Health Organization

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Acronyms

APW	Alkaline Peptone Water
CLSI	Clinical and Laboratory Standards Institute
CMV	Cytomegalovirus
COVID-19	Coronavirus Disease 2019
CSF	Cerebrospinal Fluid
EBV	Epstein-Barr Virus
EDCD	Epidemiology and Disease Control Division
EDTA	Ethylenediaminetetraacetic Acid
EMT	(Emergency Medical Team)
EPEC	Enteropathogenic *Escherichia coli*
ETEC	Enterotoxigenic *Escherichia coli*
HHV	Human Herpesvirus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
IPD	In-Patient Departments
ISO	International Organization for Standardization
IV	Intra-venous
RRC	Rapid Response Committees
RRT	Rapid Response Team
MOSD	Ministry of Social Development
NEIDL	National Essential In-Vitro Diagnostics List
NPHL	National Public Health Laboratory
OPD	Out-Patient Departments
PEP	Post Exposure Prophylaxis
PHC	Primary Health Center
PHD	Public Health Department
PPE	Personal Protective Equipment
PPHL	Province Public Health Laboratory
QC	Quality Control
RSV	Respiratory Syncytial Virus
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SORMAS	Surveillance, Outbreak Response Management System
TB	Tuberculosis
VTM	Viral Transport Medium
VZV	Varicella Zoster Virus
WHO	World Health Organization
WNV	West Nile Virus

INTRODUCTION

In the realm of infectious disease management, timely and accurate diagnosis is paramount to effective outbreak control of any infectious disease. As pathogens transcend borders and populations, the need for a robust system of clinical sample transportation becomes increasingly evident. This field manual serves as a cornerstone in this critical process, offering comprehensive guidelines and protocols tailored to the unique challenges of outbreak scenarios and acute public health events.

Nepal has laboratories at different levels of the health system that spans all seven provinces, consisting of Province Public Health Laboratories (PPHLs), district hospital-based laboratories, local hospital-based laboratories, medical college laboratories and private laboratories. At the apex, the National Public Health Laboratory (NPHL) functions as the national reference laboratory, providing technical oversight, conducting advanced diagnostics, and supporting surveillance and outbreak response. The Epidemiology and Disease Control Division (EDCD) plays a critical role in coordinating with health offices, Provincial Health Directorates, Rapid Response Teams (RRTs) at various levels, and relevant stakeholders, while ensuring timely data analysis for public health action.

At the critical juncture of any outbreak response, the swift and secure transportation of clinical specimens from the point of collection to the laboratory for analysis is pivotal. However, timely transportation of samples from the event site to testing laboratories remains a significant challenge. The logistics of sample transportation during outbreaks present a multitude of complexities, ranging from ensuring specimen integrity and chain of custody to navigating logistical hurdles in resource-constrained environments. Additionally, variations in laboratory capacity at different levels of the health system, lack of standardized coordination mechanisms, and biosafety risks further complicate the sample transport process. To address these challenges, well-defined referral pathways, a structured coordination mechanism, and trained personnel equipped with biosafety protocols are essential.

This manual acts as a guidance in the field for epidemiology, laboratory, and public health personnel. It is primarily intended to be used in conjunction with the Alert and Response Framework, Collaborative Surveillance, Rapid Response Team (RRT) and Emergency Medical Team (EMT) deployment guidelines at all levels of governance in the country. By providing a systematic approach to sample collection, packaging, labeling, and transportation, the manual ensures that specimens reach the appropriate testing facility without compromise, with a strong emphasis on maintaining the integrity of specimens while adhering to biosafety and biosecurity protocols.

Moreover, this manual is not merely a static document but a living resource, designed to evolve alongside advancements in diagnostic technology, epidemiological understanding, and best practices in outbreak response. It embodies a collaborative effort among stakeholders at local, provincial, and national levels, reflecting a shared commitment to strengthening the laboratory system and safeguarding public health in the face of emerging and re-emerging infectious threats.

SCOPE OF THE DOCUMENT

This guideline is developed to facilitate coordination between the Epidemiology and Disease Control Division (EDCD), Provincial Health Directorate, and laboratories at various levels during outbreak investigations and public health emergencies. It ensures an organized response, when Rapid Response Teams (RRTs) are deployed, and establishes standardized screening and transportation mechanisms to facilitate accurate and timely sample collection and transport. This, in turn, supports the rapid detection of pathogens, enabling prompt diagnosis and intervention during acute public health events.

This document provides a structured framework for sample collection and transport within Nepal's public health response system, including the national laboratory network. It ensures timely and efficient diagnostic services by outlining protocols for the collection, packaging, storage, and transportation of clinical and environmental samples. The guideline applies to all relevant laboratories, including public and private entities—such as the National Public Health Laboratory (NPHL), Province Public Health Laboratories (PPHLs), district-level laboratories, medical college laboratories, local hospital-based laboratories, and other key laboratories across the country.

Additionally, this guideline facilitates the development of a reliable and sustainable sample transportation system, ensuring secure and efficient transport under proper oversight. It also aims to leverage and integrate existing surveillance and laboratory networks, including disease-specific diagnostic and surveillance programs. Reference should be made to relevant guidelines and protocols governing specialized sample transport within vertical disease surveillance programs and other regulatory frameworks.

Given its broad applicability and cross cutting relevance, this document complements existing policies and guidelines, promoting coherence and harmonization across laboratory networks and public health initiatives.

COORDINATION STRUCTURE AND MECHANISM

(I) COORDINATION STRUCTURE:

The Epidemiology and Disease Control Division (EDCD) under the Department of Health Services, serves as the apex body overseeing the functioning of local, district, provincial and central Rapid Response Committees (RRCs) and Rapid Response Teams (RRTs) at all levels for public health management. During acute public health events, the EDCD coordinates with health offices, provincial health directorates and respective RRTs in the field, including health desks at Point of entry (PoE) to ensure an effective public health response. For cases requiring a national level response, the EDCD works in close coordination with the National Public Health Laboratory (NPHL) through the central Rapid Response Committee (RRC) to facilitate laboratory testing, epidemiological investigation, and overall public health response.

The National Public Health Laboratory (NPHL) serves as the apex and reference laboratory within the national coordination structure, including laboratories across different levels of the health system. The roles and responsibilities of these laboratories are illustrated in Figure 1. This structure spans all seven provinces, consisting of Province Public Health Laboratories (PPHLs), district hospital-based laboratories, local hospital-based laboratories, medical college laboratories and private laboratories. This structured laboratory system ensures timely diagnostic services at multiple levels, supporting effective public health response and disease surveillance.

(II) COORDINATION STRUCTURE DURING THE ACUTE PUBLIC HEALTH EVENTS: ROLE OF RRT IN SAMPLE MANAGEMENT

As per the Rapid Response Team (RRT) and Emergency Medical Team (EMT) deployment guideline 2079, the Rapid Response Committee (RRC) at central, provincial and local level are responsible for managing public health events including outbreaks. These committees comprise of key relevant government personnel at their respective level including those managing investigation and diagnostics. During acute public health events, these committees coordinate with each other and ensure all necessary resources including financial resources are available for effective response including sample collection, transportation and testing.

The local, district, provincial and central Rapid Response Team (RRT) is comprised of multidisciplinary teams including laboratory personnel at their respective levels. After initial rapid risk assessment, the RRT focal person along with laboratory personnel will do all necessary coordination with health offices, PHD and EDCD and arrange material

for collection, packaging and transportation of appropriate clinical and environmental samples for laboratory confirmation. The RRT should coordinate with the nearest identified laboratory for sample testing (when available). However, if the sample is being collected at the hospital level and cannot be tested at the hospital level laboratory, the RRT focal person will coordinate for transportation in nearest laboratory where testing is available.

At the central and provincial level, the necessary budget will be managed by the relevant division/branch from their annual program or other available funds. The budget required for the local Rapid Response Team's activities will be allocated at the local level.

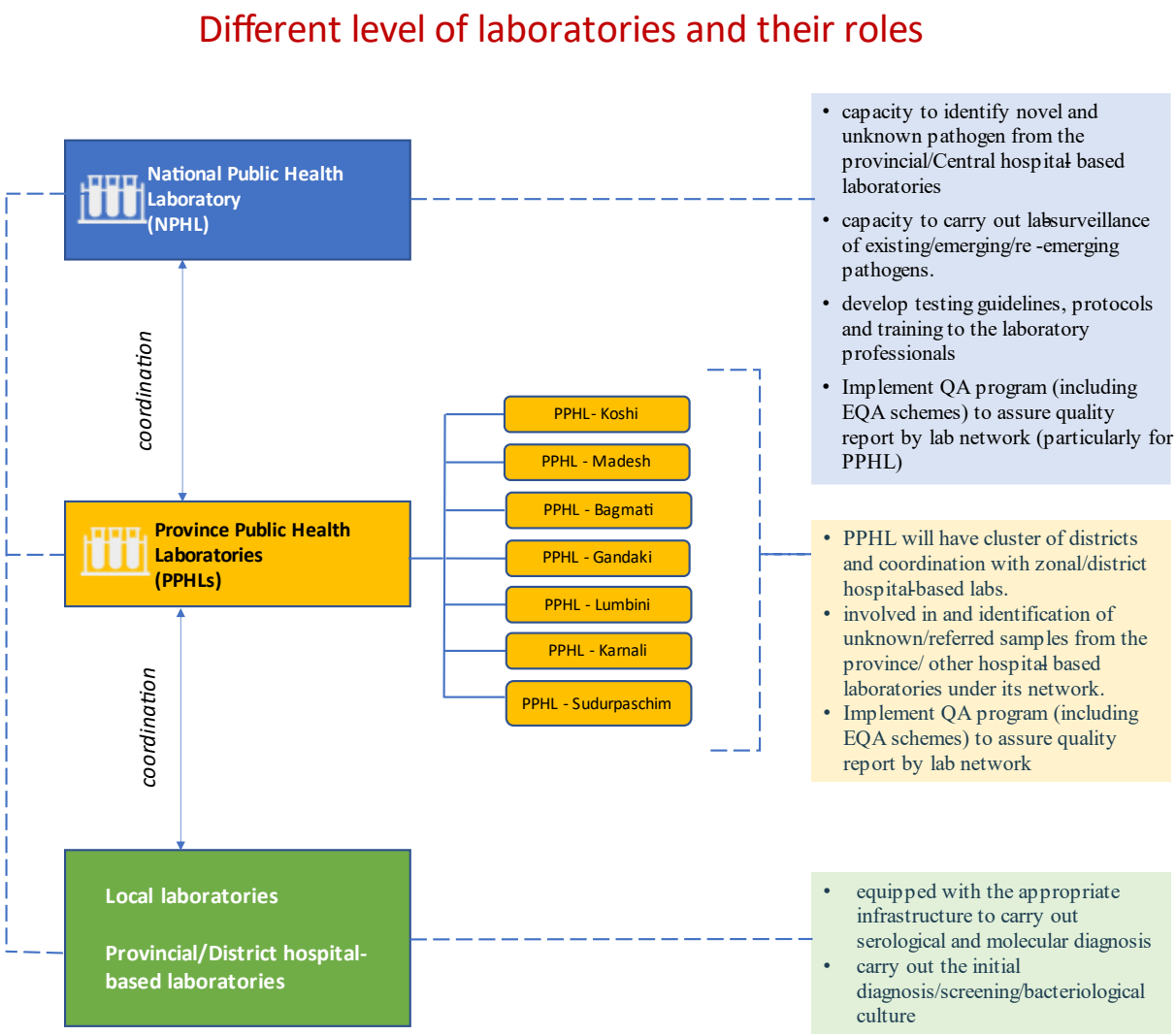

Figure 1. Different levels of laboratories and their roles

(III) DIFFERENT LEVELS OF LABORATORIES AND MECHANISMS FOR SAMPLE TRANSPORTATION:

The NPHL has state-of-the-art facilities to identify novel and unknown pathogens, sequencing capacity, biorepository, undertaking research activities and conducting surveillance of existing as well as new diseases. NPHL plays a key role in identification of unknown/referred samples from the provincial/central hospital- based laboratories, develop testing guidelines, protocols and training to the laboratory professionals and offering logistic support during outbreak investigation as applicable. As needed, the NPHL shall communicate and interact as necessary with regional World Health Organization (WHO) networks and international collaborating centers. Additionally, the NPHL is expected to provide strategic advice and share expertise to strengthen national capacity for laboratory services to support disease

The PPHLs shall carry out serology, Real Time Polymerase Chain Reaction (RT-PCR), bacterial culture, and maintain facility of storage of specimens/ isolates for referral / research purposes. Each of the PPHLs have a defined geographical coverage and in case of any outbreak / public health event, these laboratories shall do the testing in case, test is not available at local labs. These laboratories shall be involved in identification and processing of unknown/referred samples from the provincial/ other hospital-based laboratories. The province/district/medical colleges-based laboratories, including private hospitals available nearby the site of outbreak serve as local labs. These labs should have the capacity to carry out serological, microbiological (culture) and molecular diagnosis. These labs will act as the first level of labs to rule out the disease/ screening at the most peripheral areas & nearest to the site of outbreak.

Laboratory Personnel responsible for sample collection and transportation should align with three key phases during outbreak investigation:

1. Pre-deployment phase: Communication with testing lab and arrangement of logistics for sample collection, packaging and shipping
2. Deployment phase: Sample collection, labelling, packaging and shipment arrangement
3. Post deployment phase: Communication with lab to ascertain sample shipping within timeframe and in optimal conditions. Also, ensure result communication as per need

During an acute public health event, RRTs and other field investigation teams should be well equipped with tools for relevant sample collection and transportation. The diseases/agents which cannot be identified at local level and require confirmatory testing shall be referred to the Provincial or Central laboratories as appropriate. These

laboratories are also expected to carry out the initial diagnosis or screening in case of any outbreaks nearest to the site of outbreak, in coordination with respective PPHL. The lab focal person in the RRT plays a key role in preparing testing laboratories by notifying them in advance and gathering needed information on appropriate sample type, transportation conditions and other necessary details. They are responsible for arranging materials for collection, packaging and transportation of appropriate clinical and environmental samples for laboratory confirmation.

Sample transportation to local laboratories shall be facilitated by the RRT involved. In case of sample transportation to PPHL, the courier mechanism provided by PPHL can be utilized. Similarly, if a sample is being transported to NPHL for testing, the courier mechanism provided by NPHL can be utilized.

The mechanism for sample transportation from field/hospital/outbreak sites to the respective laboratory testing sites must be well-coordinated at the levels of the laboratory structure (Figure 2). Additionally, the envisioned mechanism for harmonized coordination and information sharing in real-time can be attained through (i) implementation of a digital data platform (e.g. SORMAS) at all levels for database entry and issuing reports and (ii) ensuring well-trained and adequately equipped human resources for laboratory at all levels.

The list of diseases requiring laboratory confirmation, specimen types and tests at different levels of each laboratory within the national laboratory structure is defined in the National Essential In-Vitro Diagnostics List (NEIDL) for Nepal. The focal person at each level of RRTs shall maintain an updated list of laboratories in the structure with details on focal person contact and capacity in terms of infrastructure, equipment, trained human resources, etc.

Key points on transportation mechanism:

1. The personnel conducting the field investigation is responsible for collating and submitting the epidemiological data.
2. If laboratory testing is not available *at the identified provincial health facility/medical colleges/federal hospital/ district hospitals*, RRT should coordinate with focal point of PPHL and the Province Health Directorate (PHD) and get the sample transported to provincial public health laboratory (PPHL). In situations where RRT is unable to transport samples, the courier or other existing mechanism of PPHL should be used. If laboratory testing is not available at the PPHL or further testing is required, the PPHL should facilitate transfer samples to NPHL directly from the field. This should be communicated prior with NPHL and EDCD. NPHL is responsible for needful arrangement for transportation from PPHL to NPHL, through the identified courier service or existing mechanism established by NPHL.

3. All the relevant information including test results must be communicated with EDCD, NPHL, relevant Provincial Health Directorate (PHD) and PPHL. The respective PHD/EDCD shall notify the test result and relevant information with the concerning health facility and RRT.

NOTE:

- During any event of special national concern with public health implications, Central RRT deployed at the field level will be responsible for sample collection and transportation directly to NPHL.
- Whenever critical intervention is required for sample collection, it must be done by experts. e.g. Cerebrospinal fluids (CSF) should be collected by a trained clinician of a nearby health facility in coordination with RRT.
- PPHL should define the coordination mechanism (including cost) for sample transportation from local laboratories (province/ district level, medical college and private hospitals) to PPHL as per their jurisdiction. PPHL and/or Central RRT will be responsible for deciding the best alternative testing facility based on urgency and biosafety requirement.
- The laboratory test results, concerning International Health Regulation (IHR) notification, should be notified by the testing laboratory to the designated IHR focal point (i.e. EDCD).
- In case of sample transportation within the Kathmandu valley, the sample can be directly transported to NPHL in close coordination with EDCD

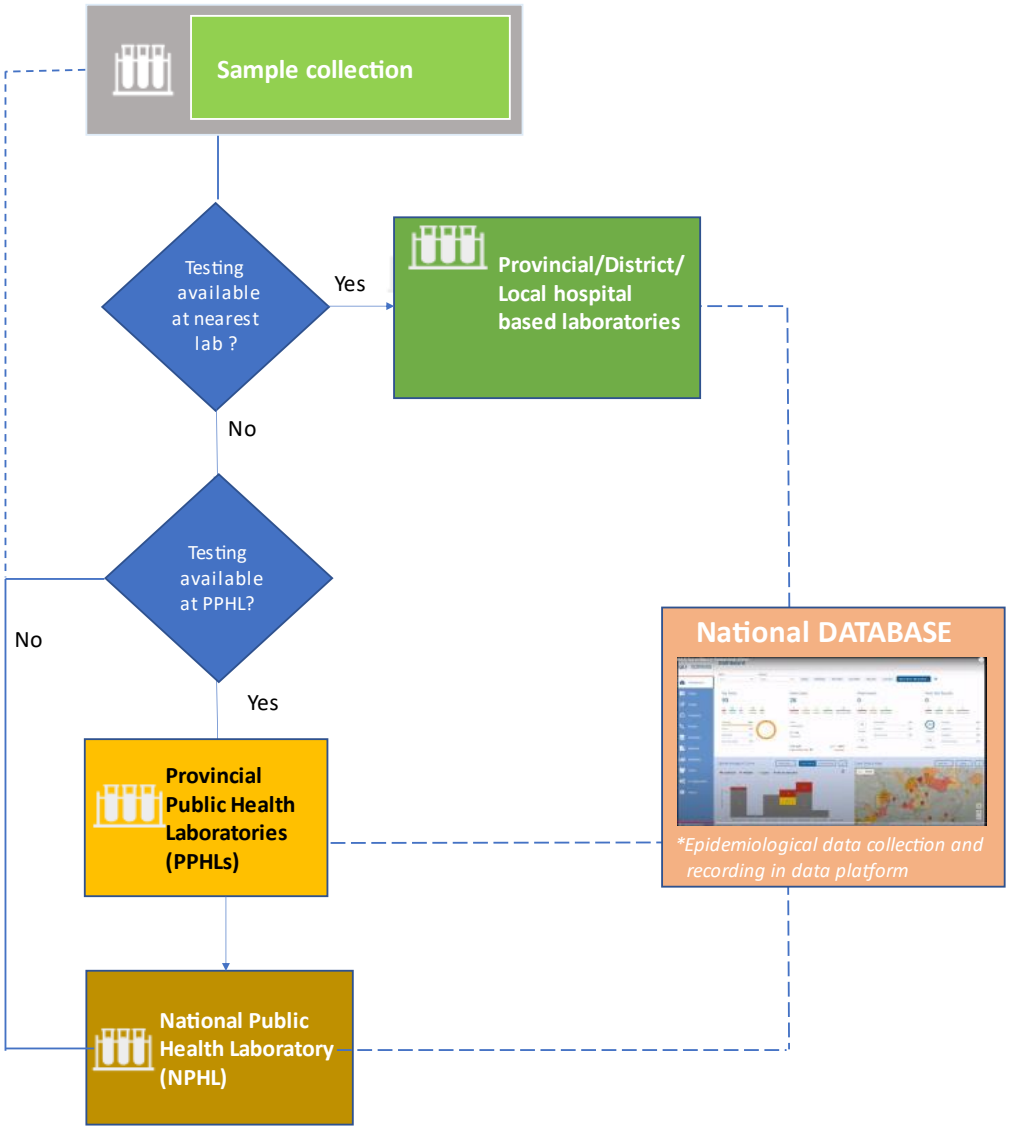

Figure 2. *Schematic diagram for laboratory sample collection, transportation, and result dissemination*

TYPES OF CLINICAL SAMPLES

According to the International Organization for Standardization (ISO) and Clinical and Laboratory Standards Institute (CLSI), a sample is defined as "one or more parts taken from a system and intended to provide information on the system" (ISO 15189:2007). Often, the term "specimen" is used in the laboratory to indicate a sample taken from the human body. The commonly collected samples are blood, urine, stool, body fluids, tissues/samples from the infected site, tissue swabs, etc. The quality of the work a laboratory produces is only as good as the quality of the samples it uses for testing. Therefore, laboratories must be proactive in ensuring that the samples it receives meet all the requirements needed to produce accurate test results.

Table 1. Clinical syndromes, aetiological agent, and selection of clinical samples

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
Acute Diarrheal Disease (ADD)/Acute Gastroenteritis (AGE)	**Bacterial:** *Shigella spp., Salmonella spp., Staphylococcus aureus* (enterotoxin producing), *Vibrio cholerae, Vibrio parahaemolyticus, Campylobacter spp. E coli* (EPEC, ETEC), *Clostridium difficile, Bacillus cereus, Aeromonas spp.*	Bacterial culture	Stool 1-2 gm/ rectal swab in Cary Blair Medium* in sterile leak proof container (for bacterial culture),	Collect specimen as soon as possible after the onset of illness (preferably within 1-4 days), and preferably before the initiation of antibiotic therapy. *Specimen in Cary Blair Medium/ bacterial culture and microscopy to be transported at room temperature. If there is a delay of 48 hours or more transport under cold chain at 2-8°C.
		Antigen detection (Cholera and Rota virus RDTs)	Stool 1-2 gm/ rectal swab	
		Real time PCR	- Rectal swab in VTM or -Fresh stool **(not in**	

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Viral: Rotavirus, Norovirus, Adenovirus, Astrovirus, Sapovirus **Others:** *Entamoeba histolytica, Giardia lamblia, Cryptosporidium spp., Microsporidium spp., Cyclospora*.		**VTM)**	Specimen for PCR and antigen detection may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.
		Microscopy for Parasite	- Fresh stool sample for microscopic examination -Wet mount (Pus cells, RBCs and trophozoites) -For specific to parasites ova/cyst: Mix stool with 10% formalin, 3 parts stool 1 part formalin (for parasites) in ambient temperature	
Acute Encephalitis Syndrome (AES)/Meningitis/ Encephalitis or Meningoencephalitis	**Bacterial:** *Streptococcus pneumoniae* *Group B Streptococcus* *Neisseria meningitidis* *Haemophilus influenzae*	Bacterial culture	- Blood for culture - CSF-for culture	Do NOT refrigerate CSF for bacterial culture. It is transported at ambient temperature, without any transport media. CSF for virology do not need transport medium,
		Microscopy	-India Ink preparation for *Cryptococcus* -Wet mount preparation for *N. fowleri* trophozoite	

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Listeria monocytogenes **Fungi:** *Cryptococcus neoformans* **Parasite:** *Naegleria fowleri* **Viral:** Japanese Encephalitis Dengue Chikungunya West Nile Virus (WNV) Cytomegalovirus (CMV) Epstein Barr Virus (EBV) Herpes Simplex Virus (HSV) Enterovirus Varicella Zoster virus (VZV) Mumps virus Parechoviruses Rabies virus	Antigen/ Antibody detection	- Clotted whole blood 4-5 ml, -CSF-0.5-1 ml	transported at 2-8°C. In case of delay store at -20°C. Acute (within a week of onset of illness) and convalescence (after 10 to 14 days after the acute sample) sera required in case of no CSF CSF for PCR, virus isolation and serology may be stored at 2-8°C for 24-48 hours, or at –20°C for longer periods.
		Real time PCR	- EDTA blood -2 ml - Clotted whole blood 4-5 ml, - CSF 0.5-1 ml **Note: CSF should be collected only by a medical officer in a health care facility*	

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Zika virus Nipah virus			
Fever with rash	**Bacterial:** *Eschar:* *Anthrax* (Bacillus anthracis)* *Scrub Typhus (Orientia tsutsugamushi)*	Antibody detection	Clotted whole blood 4-5 ml	Specimen for PCR and serology may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Virus: **Maculopapular rash:** Measles virus Rubella virus Enterovirus, Human Herpes virus (HHV) -7, 6 Parvovirus B19, Adenovirus **Vesicular rash:** HSV VZV Enterovirus, Mpox virus	Real time PCR	Nasopharyngeal / Oropharyngeal swab in VTM Clotted whole blood 4 -5 ml Soak the swab with vesicle fluid and gently rub the base of the vesicle and put into the VTM. - Crust of the vesicle in sterile dry tube - Skin scrapings from Eschar in dry tube / swan the eschar with a swab pre-wetted with sterile saline.	* If anthrax (black eschar is suspected) inform the laboratory in advance and tale specific directions on safe collection and transport of the sample)
Urethritis, Genital herpes	**Bacterial:** *N. gonorrhoeae* *Chlamydia trachomatis*	Bacterial Culture and Gram staining	Urethral/ endocervical, throat swab in Stuart's medium Smear on glass slide	Ensure to collect sample prior to antibiotic therapy whenever possible.

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Mycoplasma spp. **Viral:** HSV VZV		Urine 5-10 ml for culture	Urethral/ endocervical, swabs for culture suspected of *N gonorrhoeae* should **NOT be refrigerated.** Bed side inoculation is preferred. Please contact the laboratory in advance. Also make smear for gram stain. Urine for culture should be processed within 2 hours. **Store at 2-8 °C** if delay in transportation /processing is expected. Specimen for PCR and serology may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.
		Antibody detection	Clotted whole blood 4-5 ml	
		Real time PCR	Urethral/ endocervical swab in VTM	
Acute Lower Respiratory Tract Infections	**Bacterial**: *Streptococcus pneumoniae* *Staphylococcus aureus* *H. influenzae*	Bacterial culture	-Sputum/respiratory specimen including Broncho alveolar lavage, Pleural fluid. -Blood for culture	Transport blood for culture in culture bottles at room temperature (until placed in incubator/ automated blood culture machine)

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	K. pneumoniae & other Enterobacteriaceae *Legionella pneumophila* **Viral:** Influenza viruses SARS-CoV-2 Respiratory Syncytial Virus (RSV) Adeno viruses Enteroviruses Human coronaviruses Human metapneumovirus Parainfluenza viruses Rhinovirus Cytomegalovirus Hantavirus, Nipah virus	Antigen/ Antibody detection	- Nasopharyngeal / Oropharyngeal swab (Throat swab) in VTM - Clotted whole blood 4-5 ml	Specimen for PCR and serology may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.
		Real time PCR	- Nasopharyngeal / Oropharyngeal swab (Throat swab) in VTM	
Acute Jaundice Syndrome	**Bacterial:** *Leptospira spp.* *Salmonella spp.* **Viral:**	Antibody levels Serotyping (for Leptospira)	- Clotted whole blood 4-5 ml for serotyping	Specimen for PCR and serology may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Hepatitis viruses (A,B,C,E) CMV EBV Adenovirus		- Bile sample for culture for *Salmonella* spp.	
		Antibody detection	- Clotted whole blood 4-5 ml -	
		Real time PCR	- Clotted whole blood 4-5 ml /EDTA blood 3ml Urine 5ml (If leptospirosis is suspected).	
Parotitis	**Viral:** Mumps virus **Bacterial:** *Burkholderia pseudomallei*	Bacterial Culture	-Parotid gland duct swab in Stuart's transport medium	Swab sample for bacterial culture in transport medium can be transported at ambient temperature. Specimen for PCR and serology may be stored at 2-8°C for 24-48 hours, or at –20°C for longer periods.
		Antibody detection	- Clotted whole blood 4-5 ml	
		Real time PCR	-Oral swab (in VTM) /Saliva - - Clotted whole blood 4-5 ml	

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
Ocular (Eye) infections/ conjunctivitis	**Bacteria** *S. aureus,* *S. pneumoniae,* *P. aeruginosa,* *N. gonorrhoeae,* *Moraxella spp.,* *H. influenzae* Enterobacteriaceae **Virus** Adenovirus Enterovirus HSV VZV **Fungus** Fusarium spp. **Parasite** Acanthamoeba spp.	Bacterial Culture	-Eye/ conjunctival swab in Stuart's medium	Swab for bacterial culture in transport medium can be transported at ambient temperature Specimen for PCR and serology may be stored at 2-8°C for 24-48 hours, or at –20°C for longer periods.
		Antibody detection	- Clotted whole blood 4-5 ml	
		Real time PCR	-Eye swab (in VTM) -EDTA blood - Clotted whole blood 4-5 ml	
		Microscopy	- Corneal scrapings - (KoH mount for Fungus and Wet mount for Acanthamoeba cyst)	
Acute Undifferentiated Fever	-	Bacterial culture	Blood for culture	Preferably collect sample during the early phase of illness and before initiation of antibiotic therapy Transport blood for bacterial culture in culture bottles at
		Antigen/ Antibody detection	- Clotted whole blood 4-5 ml - Throat/nasopharyngeal swab in VTM	

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
		Real time PCR	-EDTA Blood 2 ml - Clotted whole blood 4-5 ml	room temperature (until placed in incubator) **Urine** for culture must be processed within 2 hours. **Store at 2-8 °C if** delay is expected to minimize the risk of overgrowth by contaminating organisms. Specimen for PCR and serology may be stored at 2-8°C for 24-48 hours, or at –20°C for longer periods. For serology consider collecting a paired blood sample.
		Microscopy	Blood smear Thick film Thin film To fix the dried fil by dipping the slide in methanol or other fixative and air dry	
Urinary Tract infection	Enterobacteriaceae Gram positive cocci Non-fermenting Gram Negative bacteria	Bacterial culture	5-10 ml mid-stream urine	Process within 30 minutes to 60 minutes of collection. If there is delay, may be stored at 2-8°C for 24hours.
Acute Hemorrhagic Fever	Meningococcal meningitis Dengue	Bacterial culture	5 ml whole blood for culture (in blood culture bottle)	Transport blood for culture in culture bottles at room temperature (until placed in incubator)

Disease/ syndrome	Etiological agents	Test method	Specimen required, volume/quantity	Instructions
	Cremean Congo Hemorrhagic Fever	Antigen/ Antibody detection	Clotted whole blood 4-5 ml	Specimen for PCR and serology may be stored at 2-8°C for 24-72 hours, or at –20°C for longer periods.
		Real time PCR	-EDTA Blood - Clotted whole blood 4-5 ml	

Note:

** CSF samples should be collected by a medical officer in a health care facility*

** Treating physicians will determine which sample to be collected, whenever needed. Consult the laboratory to determine the appropriate sample whenever in doubt.*

SAMPLE DATA FORM (LABORATORY TESTING REQUEST FORM)

Importance of Epidemiological Data While Filling up the Forms

Any outbreak/emergencies warrant for a timely decision for an effective response A public health professional should always use laboratory testing request form (as shown in table below) to facilitate early communication with the laboratory receiving the sample. This communication is especially crucial in outbreaks of unknown etiology to ensure that samples are collected in a manner that enables rapid laboratory testing of a variety of potential etiologic hypotheses. It is also important to remain aware that etiologic hypotheses are based on clinical signs and symptoms.

Table 2. Laboratory testing request form (minimum details required)

S. N	**Date of Sample collection** (DD/MM/YYYY)	**Sample ID**	**Name of the patient**	**Age** (month/ Year)	**Gender** (M/F/O)	**Place of Residence**	**Date of first symptom onset**	**Clinical Symptoms**	**Clinical classification of case (*suspected as)***	**Additional information**
1										
2										
3										
4										
5										
**Note: Sample ID should be labelled in the sample collecting tube or containers*										

SAMPLE COLLECTION

The proper collection and transport of samples plays a crucial role in producing high quality results. The laboratories should provide adequate training for the staffs to ensure quality in the overall process of sample collection. Samples can be categorized into three main types based on collection methods:

- Samples collected from Out-Patient Departments (OPD)
- Samples collected from emergencies/In-Patient Departments (IPD)
- Samples collected from peripheral facilities or sites

In case of outbreaks or investigations, the following points should be considered for sample collection

- Specimens obtained for culture in the acute phase of the disease, preferably prior to administration of antimicrobial drugs, are more likely to yield the infective pathogen. Avoid contamination with indigenous flora.
- For assays designed for detection of viral antigens or nucleic acid optimal specimens should be collected as soon as possible after symptom onset (within 1 to 3 days).
- For serologic diagnosis, the timing of serum collection and type of antibody detected vary for specific viruses. If testing for a virus-specific IgM assay, an acute-phase serum specimen from the first few days of illness should be obtained. For IgG assays sera from acute and convalescent phase should be obtained to demonstrate a rise in antibody concentration.

The initial step prior to actual sample collection is obtaining the sample request. The identification of patients, specifying the test requested, date and time for sample collection, source of sample, etc. are required before sample collection in most cases.

SAMPLE COLLECTION REQUIREMENTS

The process of sample collection mainly depends on the required laboratory test to be performed. The main steps in the sample collection procedure include:

- *Patient Identification:* The proper identification of the patient should be made before collecting the sample. This can be done by asking the patient's name or looking at details on the requisition form.
- *Preparation of the patient:* As per the requirement of test to be performed, the patient should be advised to prepare accordingly. For example: early morning sputum or fasting for blood glucose or hormonal level, etc.
- *Type of sample:* Depending on the requisition of test, the sample should be collected. The sample can be either whole blood, plasma, serum, body fluids or urine, saliva, etc.
- *Type of Container:* The container for the sample is often very important, as it will affect volume and any additives needed such as anti-coagulants and preservatives
- *Proper labelling of sample*
- *Proper use of safety measures:* This includes the proper use of PPEs and other measures to protect health workers from infection by the pathogens.

The collection of samples during emergencies or in the field of epidemiological studies requires special attention to proper patient identification and accurate labelling. In addition to meeting the above-mentioned requirements, handling highly infectious agents like COVID-19 or other pathogens, the latest/updated international and national guidelines should be followed. Furthermore, when patients are required to collect their own samples, such as stool specimens, they should be advised on the proper sample collection procedures.

SAMPLE LABELLING

Labelling has an important role to play throughout the sample transportation pathway. Every sample should be clearly labelled with the patient's first and last name, a unique identification number, requested test, the time and date of sample collection, etc. During outbreaks, the original documents remain with the investigation team, consolidated for analysis and future reference. Additionally, a laboratory request must also be completed for each specimen and sent alongside it, containing the following information:

The routine samples should be packed safely to avoid spillage. For the transportation, all the clinical samples should be packed in triple layered container packing as per the national and international guidelines.

SAMPLE HANDLING AND STORAGE

All types of samples should be preserved and transported at the recommended temperature. Proper packaging must be ensured for the safety of personnel from the collection site to laboratory and preserve the specimen, even if damage occurs during transit. The following points should be considered for specimen storage:

- Once the specimen is collected, it should be transported to the laboratory as soon as possible.
- Specimens for culture should be kept in appropriate transport media/Viral Transport Medium (VTM) (classical) at the recommended temperature and transported to laboratory at the earliest possible. This ensures bacterial viability while minimizing overgrowth of other microorganisms. With the exceptions of urine, CSF and sputum (exception of particularly cold-sensitive organisms such as meningococcus, and pneumococcus), most specimens may be kept at ambient temperature if the specimen is processed within 12 hours.
- Viability is not required for antigen/antibody and nucleic acid detection. Viral RNA/DNA detection specimens should be transported in 2-8° C within 7 days. Consider avoiding degradation of the viral RNA or DNA.

SAMPLE PACKAGING

Standardized (triple layer) packaging methods and materials ensure safety of personnel and specimen integrity, even in cases of damage during transport. Properly labelled specimens must be accompanied by laboratory request forms. Specimens must be packaged, labelled, and transported in compliance with national guidelines and international regulations for infectious materials.

- *Primary packing:* The specimen is in the labeled primary container. The primary receptacle should be leak-proof, sealed properly, wrapped in absorbent material (e.g., cotton wool) and appropriately labelled as content. For example, the specimen tube. It should be placed in a secondary receptacle.
- *Secondary packing:* The Secondary receptacle should be durable, watertight, leak proof bag such as sealed plastic bags. The primary packing should be secured in secondary packing with a suitable absorbent material such as cotton, enough to absorb all the specimen in case of any leakage. A biohazard label and the laboratory request form sealed in a plastic bag should be taped to the outside of this secondary container.
- *Outer packing:* The outer packing should be rigid enough to protect from physical damage and water while in transit. It should have a resistant, high density external cover (e.g., metal, wood, or fiberboard), shock-absorbent padding on the inside, and a tight-fitting lid. The outer package must be leak-proof and well insulated, and can contain ice, cold packs

or dry ice when required. For example, thermocol box or rigid plastic container.

The rigid outer package is placed within an outer carton of double-ply corrugated cardboard or plastic, and a biohazard label is applied. The specimen carriers and ice packs can be reused after proper disinfection. Examples of appropriate packaging are provided in figure 3 below.

Steps:

1. Properly label the sample.
2. Wrap the primary container with absorbent material.
3. Place the primary container inside a leakproof secondary container.
4. Place the secondary container into a tertiary container (ice box).
5. Add a gel pack and the wrapped sample collection form to the box.
6. Close the tertiary container.
7. Seal the tertiary container.
8. Attach the relevant information, including consignee and contact details, to the outside of the box.

Figure 3. *Different stages of sample packaging*

Note: Place appropriate hazard label on the outer package (Example: infectious hazard label).

SAMPLE TRANSPORTATION

In routine procedures, samples are typically collected and processed within laboratories or health facilities, eliminating the need for transportation. However, during outbreaks or when samples require further processing and testing at provincial or national laboratories, careful management is essential. Transport protocols must prioritize maintaining sample integrity, temperature control, and adherence to regulations on packaging and labeling. Samples should be triple-packaged and transported via various means, including roads and airways, while adhering to national and international biosafety regulations. **Category A or B** classified samples should have appropriate labeling and transportation as per standard norms.

Once the clinical specimen has been collected, it should be transported to the laboratory as soon as possible under the following details:

Table 3. Sample transportation form

Type of sample	Purpose / Test	Transportation media used	Transportation temperature	Remarks

Maintenance of transit temperature

Once the clinical specimen has been collected, it should be transported to the laboratory as soon as possible. For timely diagnosis, delays should be avoided during specimen transportation.

- **2-8 °C:** In case of transportation delays, samples should be stored at 2-8°C and transported to PPHL/NPHL within 72 hours of collection. The transport box should be fitted with a minimum of 4 ice packs, or more if room is available, around the secondary container. This will maintain refrigeration for 2-3 days. If available, a cold chain monitor should be inserted.
- **-20 °C:** Use 2 kg of dry ice within the insulated outer package, which must permit the release of carbon dioxide gas to prevent explosions. This will keep the specimens frozen for 1-2 days.

(Instruction for post-collection of specimens)

- *Samples should be collected and transported in appropriate containers to maintain their stability and to avoid rejection*
- *Please check the containers for any defect /leakage*
- *Aseptic techniques should be followed while collecting sample to prevent contamination to the sample as well as patient.*

Hence, the sample should be handled carefully before, during and after the transport process to ensure compliance with all national and international regulations.

BIOSAFETY AND BIOSECURITY

Safety and decontamination procedures protect the specimen collector and colleagues, laboratory personnel, and the patient from risks associated with specimen collection. Ensure that protective clothing is worn and safe work practices are followed to reduce exposure to infectious materials. A first aid kit is also essential and should be readily accessible at the site of specimen collection.

- Use latex or nitrile gloves when taking and handling specimens.
- Dispose of gloves between patients and replace with a fresh pair. Do not attempt to clean and reuse gloves as this may promote the spread of pathogens from patient to patient.
- Wear protective clothing (gown, coat or apron) when collecting samples.
- Discard used needles directly into sharps box, without recapping them.
- Work areas and surfaces should be organized and disinfected with 1% household bleach daily or with a change in collection team. Use 10% bleach to clean up spills after wiping the surface clean. Personnel carrying out cleaning or decontamination should wear a protective coat and thick rubber gloves.
- Contaminated non-disposable equipment or materials should be soaked in 1% household bleach for 5 minutes. Before using wash in soapy water and sterilize if necessary. Heavily soiled disposable items should be soaked in 10% household bleach before incineration or disposal.
- Viability is not required for antigen/antibody and nucleic acid detection. Viral RNA/DNA detection specimens should be transported in 2-8^{o} C within 7 days. Consider avoiding degradation of viral RNA or DNA during storage and transport.
- Specimens for antigen or antibody detection may be stored at 2-8°C for 24-48 hours, or at –20°C for longer periods.
- For timely diagnosis delay should be avoided during transportation of the specimens
- Ensure appropriate packaging and labelling as required for transportation of infectious substances.
- Ensure biosecurity while the sample is being transported and biosafety during transportation.

BIOMEDICAL WASTE MANAGEMENT

(including spill management and PEP for needle stick injury)

Bio-medical waste refers to any waste generated during the diagnosis, treatment or immunization of human or animals, or any research activities pertaining thereto or in the production or testing of biological matter, or in health camps. Biomedical waste is a heterogeneous mixture, which is very difficult to manage as such. But the problem can be simplified, and its dimension reduced considerably if a proper management system is planned.

Improper and inadequate biomedical waste management can cause environmental pollution, growth, and multiplication of vectors like insects, rodents, and worms and may lead to the transmission of diseases such as Typhoid, Cholera, Hepatitis, and AIDS through injuries from syringes and needles contaminated with human derived products. Hence, it requires specific treatment and management prior to its final disposal.

International and national guidelines should be followed, where and when relevant. Some relevant documents are as follows:

1. Laboratory Biosafety manual, WHO, 4th edition, 2020
2. National Health Care Waste Management Standards and Operating Procedures - 2020 Standard Operating Procedure (Sop) For Laboratory Waste Management

STEPS IN WASTE MANAGEMENT

Waste collection and segregation

Segregation at source is essential to prevent non-hazardous waste from being contaminated by hazardous materials. Mixed waste must not be segregated but treated according to the contaminant's properties. Non-hazardous waste should be segregated based on disposal routes, such as recycling or municipal waste.

Categories of waste is basically done into two broad groups, i.e., Risk waste and non-risk waste. Subcategories of risk and non-risk waste are also done based on properties and hazard nature of waste.

Waste collection should be done in color coded containers before treatment and final disposal.

Categories of waste and respective color codes are as follows:

Table 4. Waste category and color coding

Waste category, symbol and label		Colour of container
Non-risk HCW	Biodegradable	**Green**
	Non-biodegradable	**Blue**
Risk HCW	Pathological waste **Danger! Pathological waste** 	**Red**
	Sharps Waste **Danger! Sharps waste** 	**Red**
	Infectious Waste 	**Red**
	Pharmaceutical waste	**Red**
	Cytotoxic Waste 	**Red**
	Chemical Waste **Danger! To be discarded by authorized staff only**  	**Yellow**
	Danger! Radioactive Waste	**Black**

TREATMENT

Risk waste should be treated to render it safe for disposal. Various treatment methodologies are available. Appropriate methods should be selected based on type of waste as well as the availability of the method.

A few of the recommended methods are:

1. Autoclaving
2. Chemical treatment
3. Microwave
4. Landfill
5. Encapsulation

TRANSPORTATION

In case treatment facilities/ option is not available in the field, waste can be transported to the nearby health facility or the site where it can be further treated. During transportation of risk waste, all needed precautions should be implemented in order to ensure the safety of workers, the public and environment.

DISPOSAL

All the waste materials are to be disposed of after decontamination/ waste treatment, as per need. Disposal of waste then after can be done as per existing regulations and guidelines. Some of the methods of disposal may be:

- Disposing as Municipal waste,
- burial and
- landfill

Spill management (Biological sample)

This section covers the proper cleaning of spills involving biological materials in the laboratory.

RESPONSIBILITY

It is the responsibility of the laboratory personnel handling the infectious material to manage the spill of biological samples in their laboratories or workspace.

REQUIREMENTS

Spill Kit

A spills kit should contain all the things needed to clean a blood or body substance spill, such as:

1. Written spill clean-up procedure
2. Disposable gloves, protective clothing disposable plastic apron and safety goggles
3. Face mask (for protection against splashing and aerosols).
4. Tape or marking pencil to mark off spill area
5. Appropriate freshly prepared chemical disinfectant (check expiry date and dilution)

   5-10% sodium hypochlorite (bleach) are most common
6. Absorbent material (paper towel)
7. Disposal bags – leak proof, autoclavable and labelled with biohazard tags
8. Sharps collector and forceps for picking up broken glass or sharps

CLEAN UP PROCEDURES

If someone is injured and contaminated, provide first aid assistance if possible and ask an uncontaminated co-worker to call for medical assistance. Do not take a contaminated person out of the laboratory prior to proper decontamination. If a person is contaminated but not injured, have them remove contaminated clothing, and assist the person use an emergency shower or eyewash as needed.

- If the spill is in the open laboratory, alert people in the vicinity and evacuate the laboratory immediately. The last person closes the doors. Do not re-enter for at least 30 minutes so aerosols can be cleared to minimize the risk of inhalation exposure. Do not spread

contamination beyond the laboratory by staying as close to the laboratory as possible and disinfecting shoes.

- Place contaminated personal protective equipment to biohazard bag for disposal as biomedical waste. Wash your hands!
- Post-spoil signs and keep personnel not involved in cleaning up the spill away from the area.
- Put on clean gloves and impervious gown or lab coat; (Put on clean disposable face mask and protective eyewear if splashing of fluids is anticipated). For risk group 2 agents: Wear a lab coat/apron, eye protection and disposable gloves. For risk group 3 agents: Wear a disposable gown, shoe covers, a respirator, eye protection and gloves.
- Remove contaminated sharps from the spill using forceps or tongs, NOT your hands!
- Cover the spill with paper towels or other absorbent material. Take care to avoid making the spilled material splash or spray.
- Pour a freshly prepared 1% sodium hypochlorite solution around the edges of the spill and work inward to the center. Allow 15-20 minutes for the bleach to kill the organisms.
- Use additional paper towels to wipe up the spill, working from the edges into the center.
- After initial cleanup, flood the spill area with 1% sodium hypochlorite and let stand for at least 15-20 minutes.
- Use paper towels to absorb spill, then wipe-down with clean paper towels soaked with bleach.
- Disinfect any equipment, walls, or other areas likely to have been splashed by the spill.
- Pour/wipe with 70% isopropyl alcohol or water to clean and neutralize the corrosive action of sodium hypochlorite solution.
- Discard paper towels into a yellow biohazard bag. Discard plastic/rubber items to red biohazard bag. Discard glass items to blue biohazard bag.
- Thoroughly wash your hands and any contaminated skin with soap and water.
- Notify a biosafety officer if the spill is large, involves risk group 3 materials, a toxin, or if an injury or inhalation exposure may have occurred.

RECORDING OF SPILLS

Maintain a log for each spill that occurred in the laboratory. The logbook should include details about the spill (who, when, and how it occurred), the nature and associated risks, personnel exposed to the spill, individuals involved in spill cleaning, and a checklist for evaluating cleaning efficacy.

FOR NEEDLE STICK INJURY

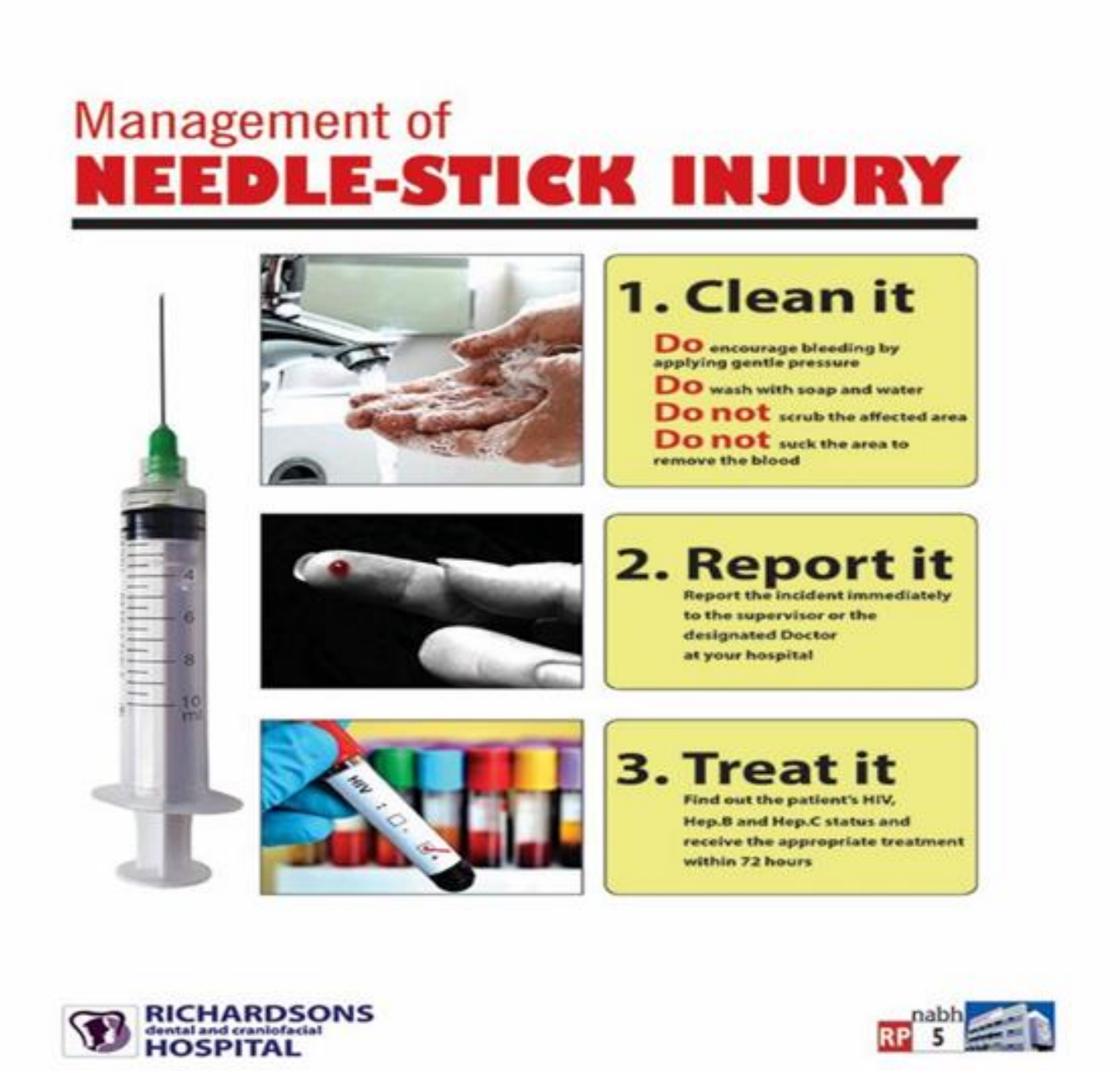

Figure 4. *Management of needle stick injury*

LIVING GUIDANCE1 FOR INTEGRATING GENDER, EQUITY AND HUMAN RIGHTS (GER)

Gender, equity and human rights (GER) are crucial considerations in health and clinical management. Individuals, whether men, women, girls, boys, or those of diverse gender identities, abilities, and sexual orientations, experience disparities in health status, exposure to risk and vulnerability, access to and use of services, health-seeking behavior, experiences in health care settings, and health and social outcomes due to their biological and social standing in the society. Health inequities manifest through differential exposure, vulnerability, access, health outcomes and consequences, so it is very important to recognize these aspects and provide health services from gender, equity, and human rights perspectives. During the process of clinical sample collection and transportation, the following aspects should be strongly considered in both field and healthcare facility settings.

1. PROVIDING RESPECTFUL CARE TOWARDS ALL PATIENTS

Naturally, we envision a relationship between patients and service providers characterized by care, empathy, compassion, support, trust, confidence, and empowerment, as well as gentle, respectful, and effective communication that enables informed decision making. When treating patients who are confirmed cases or have signs/symptoms, health workers/providers need to be mindful of diversity, providing fair treatment, respecting, and protecting the rights of each individual, including those from disadvantaged or excluded population groups.

Health workers/providers need to:

- Demonstrate equitable and fair treatment/ behavior irrespective of an individual's age, sex, caste, ethnicity, socioeconomic status, education, sexual orientation, family/cultural background, disabilities, or any other characteristics.
- Respect the right to information, right to participation with informed consent and refusal; right to confidentiality, privacy, dignity, choices/preferences, equitable care; and self-determination; right to freedom from harm, ill

Managing social stigma - Dos and Don'ts

DO – Use respectful and dignified verbal, and body language
Don't – Use offensive verbal and body language. *
DO - talk about the disease as a public health issue that concerns all people.
Don't - attach locations or ethnicity, sexual identity and orientation to the disease e.g., "Chinese Virus".

DO - talk about people "acquiring" or "contracting" communicable disease (e.g. Nipah, SARS-COV-2m etc.)
Don't talk about people "transmitting the disease" "infecting others" or "spreading the virus" as it implies intentional transmission and assigns blame.

DO - speak accurately about the risk from the disease in circulation, based on scientific data and latest official health advice.
Don't - repeat or share unconfirmed rumours.

DO - talk positively and emphasise the effectiveness of prevention and treatment measures.
Don't - emphasise or dwell on the negative, or messages of threat.

DO - emphasise the effectiveness of adopting protective measures to prevent acquiring the disease, as well as early screening, testing and treatment.

For more information:
CDC Centers for Disease Control and Prevention

[1] WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update

treatment and discrimination; and right to timely healthcare and to the highest attainable level of health.

- Avoid unintended biases towards women, girls, (with or without disabilities) or any clients based on their identity and socioeconomic backgrounds.
- Be aware of social stigma and practice the **Dos and Don'ts**. Refer to the box - there are some dos and don'ts on language[2].
- Be culturally sensitive and appropriate to age, gender, disabilities, sexual identity and orientation etc.
- Be aware of gender-related biases in access to information, health seeking behavior, service provision etc. and be non-judgmental.
- Promote evidence-based messaging that provides information on the importance of sample collection and encourage seeking health care if experiencing disease symptoms.

2. RESPONDING TO GENDER-BASED VIOLENCE (GBV)

Available evidence points to significant increase in GBV in any emergency situation, and it has been exacerbated in COVID-19 situation, and this has alarmed all actors working against GBV.

What is gender - based violence (GBV)?

Gender based violence refers to harmful acts directed at an individual based on their gender. It is rooted in gender inequality, the abuse of power and harmful norms. GBV is a serious violation of human rights and a life-threatening health and protection issue. GBV is committed in many forms such as physical, emotional/psychological, sexual, cultural/social, economic or any kind that endangers the safety, health and well-being of an individual.

Domestic Violence refers to violent or aggressive behavior within home involving intimate partner and immediate family members.

FIVE ACTIONS FOR HEALTH WORKERS/PROVIDERS TO RESPOND TO GBV

- ✓ Be aware of the increased risk and health consequences of GBV in the context of emergencies.
- ✓ Recognize the signs and know when and how to ask about violence.
- ✓ If violence is disclosed, act to provide timely care for physical, sexual, reproductive and mental health.
- ✓ If violence is disclosed, provide **First-line** support and medical care to survivors.
  The first-line support is most important, and it involves 5 simple tasks of **LIVES:**
  - LISTEN: listen closely, with empathy, and without judging.
  - INQUIRE: assess, identify and respond to a person's various needs and concerns.
  - VALIDATE: show that you understand survivor's experience, feeling and believe them.
  - ENHANCE SAFETY: discuss a plan to protect the survivor from further harm if violence occurs again.
  - SUPPORT: support them by helping them connect to information, services, and social support.
- ✓ Share information about available support, identify **referral pathways** and refer to other essential services.

Health facilities can identify and provide information about locally available (e.g. hotlines, one-stop crisis management centers, shelters, psychosocial counselling) for survivors, including details on opening hours, contact information, and whether services can be offered remotely, and establish referral linkages. It is important to understand that marginalized groups, including

[2] Disability inclusive guidelines in English FINAL

individuals with disabilities, may face additional risks and have specific needs. For issues concerning women and girls with disabilities, the National Federation of Disabled Nepal (NFDN) can be informed, approached, or contacted to report any such cases.

Note: *Safety, respect, confidentiality, and non-discrimination in relation to GBV survivors and those at risk are vital considerations at all times.*

3. CONSIDERATIONS FOR MANAGERS

Studies[3] shows globally, women make up 70 per cent of the health workforce, especially as nurses, midwives and community health volunteers, and account for the majority of service staff in health facilities as cleaners, launderers and caterers. However, they are not proportionately represented at senior, managerial and decision-making levels in health - only 25% hold senior roles[4].

This scenario also exists in Nepal. Despite the large number, women are often underrepresented in decision-making roles during health emergencies. Further, women are still paid less than their male counterparts for similar work, hold fewer leadership positions in the health sector, and experience lower job security and social protection. Masks and other protective equipment designed and sized for men leave women at greater risk of exposure. Additionally, the lack of adequate attention to the menstrual hygiene needs of women health workers during long shifts is an added workplace-related challenge.

So, health managers need to be aware of these gender-related issues and ensure that the needs of women health and care workers are prioritized and fulfilled. This means:

- The health and care workers have access to women/gender-friendly personal protective equipment (PPE) and menstrual hygiene products, i.e., the different sizes and the design of the PPE needs to be made available and accessible considering the feminine and menstrual hygiene need.
- Flexible working arrangements are available to balance the burden of care, especially for pregnant and breastfeeding mothers.
- Policies and actions are implemented to foster women health workers' increased participation in leadership and decision-making roles.
- Equal treatment and pay, paid leave, including paid sick leave, and other social protection measures are ensured to women health workers in the public and private sectors.

4. MANAGING DISAGGREGATED DATA (SEX AND AGE DISAGGREGATION OF DATA)

[3] UN Women | Explainer: How COVID-19 impacts women and girls

[4] DELIVERED BY WOMEN, LED BY MEN: A GENDER AND EQUITY ANALYSIS OF THE GLOBAL HEALTH AND SOCIAL WORKFORCE. Human Resources for Health Observer Series No. 24

Experiences from other outbreaks, epidemics and pandemics indicate that women and girls (with or without disabilities), men who have sex with Men (MSM), and individuals from marginalized groups often face specific and disproportionately high economic, health, and social risks due to deeply entrenched inequalities, social norms, and unequal power relations. Therefore, understanding the gender-differentiated impacts during public health events/disease outbreaks through sex and age, caste/ethnicity, disability disaggregated data is fundamental to policy and programme responses that can reduce vulnerable conditions and build the agency of girls and women and marginalized groups placing gender, equity, and rights at their center.

To manage the disaggregated data, the sample collection form should include at least sex, age, disabilities, caste/ethnicity, co-morbidities, and health care worker status, and this should be reported in regular reporting system. Analysis by this disaggregation should be prioritized by the health facilities and higher levels to identify any gaps and develop priorities for interventions. The same can be used to analyze health inequities among different vulnerable groups, and to review, take appropriate actions and report periodically.

ANNEX 1: SPECIMEN COLLECTION INSTRUCTIONS

SN	Specimen Type	Collection Instructions	Required list of consumables
1	**Collection of Blood**	Detection of viruses in **plasma or leukocyte** fractions requires the use of anticoagulant EDTA for obtaining plasma for nucleic acid testing (heparin inhibits many nucleic acid amplification enzymes). **Whole blood** used for nucleic acid amplification must be processed to remove serum (heme and metabolic precursors of heme inhibit DNA polymerase. **Whole Blood** is collected for culture in blood culture bottles. **Serum i**s used for serologic testing and for nucleic acid assays. **Phlebotomy procedure** 1. Label all the necessary tubes and label them. 2. Select the appropriate vein for venipuncture. 3. Consider following while site selection: • Extensive scarring or healed burn areas should be avoided. • Avoid areas of hematoma. • If an IV is in place, samples may be obtained from the other arm or below but NEVER above the IV site.	○ Blood Collection Tube (EDTA tube and Plain tube) ○ Blood culture bottle ○ Butterfly needle ○ Hypodermic Needle (21 to 23 gauge) and Syringe (5 ml: Closed system/ open system. ○ Vacutainer tube holder ○ Torniquet ○ Chlorhexidine Alcohol swabs or individually packaged 70% isopropyl alcohol. ○ Cotton balls or gauze pads ○ Adhesive bandage ○ Gloves ○ Sharp Disposal container ○ Biohazard bag ○ For adult blood culture - 10 ml aerobic bottle and 7 ml anaerobic blood culture (automated

SN	Specimen Type	Collection Instructions	Required list of consumables
		• Do not obtain specimens from an arm having a cannula, fistula, or vascular graft. 4. Apply the tourniquet 3-4 inches above the collection site. Never leave the tourniquet on for over 1 minute. If a tourniquet is used for preliminary vein selection, release it and reapply after two minutes. 5. Clean the puncture site by making a smooth circular pass over the site with the 70% alcohol pad, moving in an outward spiral from the zone of penetration. Allow the skin to dry before proceeding. Do not touch the puncture site after cleaning. 6. Perform the venipuncture as follows: • Attach the appropriate needle to the hub by removing the plastic cap over the small end of the needle and inserting it into the hub, twisting it tight/ Fix the BD (Becton Dickinson) eclipse needle to it • Remove the plastic cap over the needle and hold the level up. • Pull the skintight with your thumb just below the puncture site.	system) or BHI broth (45 ml, conventional culture) ○ For Pediatric Blood Culture (Peds plus blood culture bottle) or BHI broth (27 ml, conventional culture)

SN	Specimen Type	Collection Instructions	Required list of consumables
		• Holding the needle in line with the vein, use a quick, small thrust to penetrate the skin and enter the vein in one smooth motion. • Holding the hub securely, insert the first vacutainer tube • (following proper order of draw) into the large end of the hub penetrating the stopper. Blood should flow into the tube. *https://microbiologynote.com/types-of-blood-collection-tubes-and-their-uses/* • After blood starts to flow, release the tourniquet, and ask the patient to open his or her hand. • When blood flow stops, remove the tube by holding the hub securely and pulling the tube off the needle. If multiple tubes are needed, follow the **proper order of draw** to avoid cross contamination and erroneous results as follows: 7. Each coagulation tube (light blue top) should be gently inverted 3-4 times after being removed from the hub. Red (no anticoagulant) and gold tops should be inverted 6-8 times. All other tubes containing an additive should	

SN	Specimen Type	Collection Instructions	Required list of consumables
		be gently inverted 8-10 times. **DO NOT SHAKE OR MIX VIGOROUSLY.** 8. Place a gauze pad over the puncture site and remove the needle. 9. Immediately apply slight pressure. Ask the patient to apply pressure for at least 2 minutes. 10. When bleeding stops, apply a fresh band aid. • Properly dispose of hub with needle attached into a sharp container	
	Adult (Blood for Culture)	1. Don gloves. 2. Clean the puncture site with a chlorhexidine-alcohol swab in concentric circles. • If the site needs to be touched again before the venipuncture, the phlebotomist may clean their finger with a chlorhexidine-alcohol swab. 3. Allow the site to dry completely. 4. Remove cap of bottle(s) and disinfect the top(s) of the bottle(s) with an alcohol swab and allow to dry. • Do not touch or otherwise contaminate the top of the bottle before collection.	

SN	Specimen Type	Collection Instructions	Required list of consumables
		5. Using a butterfly needle (recommended method) insert the needle into the vein and collect 10 mL into the aerobic bottle first, and then 7 mL into the anaerobic bottle. • If using a syringe, insert the needle into the vein and collect 17 mL of blood. • Inoculate 10 mL into the aerobic bottle and then 7 mL into the anaerobic bottle. (automated system) or BHI broth. • Do not introduce air into the anaerobic bottle. • Do not change needles to fill bottles. 6. Swirl bottles to avoid clotting. 7. Place pressure on the collection site with cotton or gauze after removing needle and make sure bleeding has stopped before bandaging. 8. Safely discard collection needles. 9. Label container with two identifiers, source (site), date (time) of collection. • Do NOT cover bottle barcode. 10. Repeat steps above **on a second site. (*Recommended to collect from 2 different sites)***	

SN	Specimen Type	Collection Instructions	Required list of consumables
	Pediatric (Blood for Culture)	1. Don gloves. 2. Clean the puncture site with a chlorhexidine-alcohol swab in concentric circles. • If the site needs to be touched again before the venipuncture, clean finger with a chlorhexidine-alcohol swab. 3. Allow the site to dry completely. 4.Remove cap of Pink Peds Plus blood culture bottle (automated system) or BHI broth and disinfect the top of the bottle with an alcohol swab; allow drying. • Do not touch or otherwise contaminate the top of the bottle before collection. 5. Using a butterfly needle (recommended), insert the needle into the vein and collect the appropriate volume • If using a syringe, insert the needle into the vein and collect the appropriate volume and inoculate the bottle. • Do not change needles to fill bottles. 6. Swirl bottle to avoid clotting.	

SN	Specimen Type	Collection Instructions	Required list of consumables
		7. Place pressure on the collection site with cotton or gauze after removing needle and make sure bleeding has stopped before bandaging. 8. Safely discard collection needles.	
2.	**Serum (separation from blood)**	1. As described above draw 5 ml of venous blood and transfer to a screw cap tube without anti-coagulant. Alternatively, blood may be collected directly into Vacutainer. 2. Let the blood specimen clot for 30 minutes at an ambient temperature. 3. Centrifuged in the laboratory at low speed (1000g for 10 minutes) 4. Separate the serum aseptically from the clot using pipette 5. Transfer equally to 2 plastic screw cap tubes. Secure the caps tightly. 6. Sera may be stored at 2-8°C for up to 10 days. If a testing delay is expected, store at -20°C	Centrifuge machine with rotors and stopper PPE (gloves, masks, aprons) Marker label

SN	Specimen Type	Collection Instructions	Required list of consumables
		(*Note: If a viral hemorrhagic fever is strongly suspected, samples should only be processed in properly equipped, specialized laboratories.)* 7. If separation on site is not possible, or is inadvisable for safety reasons, store blood at 2-8°C. Prevent such unseparated samples from excessive vibration while transporting. Unseparated blood samples **should not be frozen.**	
3.	**Cerebrospinal Fluid**	**(Must be collected by a physician or trained personnel when clinically indicated)** 1. Disinfect site with 2% iodine tincture. 2. Insert a needle with a stylet at L3-L4, L4-L5, or L5-S1 interspace. 3. On reaching the subarachnoid space, remove the stylet and collect CSF fluid sequentially into four calibrated sterile tubes with caps and label. 4. If only one tube of CSF is collected, it should be submitted to microbiology first.	• Sterile CSF collection tube • Collection trays • Needle and syringe • Sterile gauze and adhesive bandage • Label and markers • PPE (gloves, mask, apron) • Disinfectant (2% iodine tincture) • Sharp disposal bag • Biohazard bag

SN	Specimen Type	Collection Instructions	Required list of consumables
		5. Label container with two identifiers, source (site), date (time) of collection and hand deliver with requisition to laboratory.	
4.	**Faeces/stool**	1. Advise NOT to take any laxatives, enemas, or antibiotics for one week before the stool collection unless permitted by physician. 2. Empty bladder. 3. Collect stools in a dry, clean, empty disposable plastic container. i. Do NOT let urine or water mix with the stool specimen ii. Do NOT use toilet paper to collect stools 4. Using the plastic paddle attached to the lid, collect small portions from each end and middle of the stool, especially sampling from areas of the stool which contain mucus or blood.	• Sterile leak proof and wide mouthed container free from any kind of additives and preservatives • Transport Media such as Alkaline Peptone water (for suspected Cholera), Selenite F Broth, Cary Blair Media • Biohazard Media • Gloves • Biohazard bag

SN	Specimen Type	Collection Instructions	Required list of consumables
		5. Place solid stool (about the size of a walnut) into appropriate transport media (for bacterial culture). Stool for nucleic acid test need not be placed in VTM. 6. Replace the cap on the container securely. 7. Ensure the outside of the container is not contaminated with stool. 8. Wash hands with soap and water. 9. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to the laboratory. 10. Individual case history, symptomatology and epidemiology (food and drink if possible 3 days prior to onset to be mentioned in test requisition form.	
5.	**Rectal swab**	1.Carefully insert swab ~1 inch (2.54 cm) beyond anal sphincter. 2. Gently rotate the swab to sample anal crypts. 3. Place the swab in transport media (appropriate bacterial, viral media) as required.	• A sterile swab with a flexible shaft and a soft tip • Water based lubricant. • Collection tube or Transport media (Cary -Blair, Stuart, buffered glycerol-saline) • Biohazard bag • Gloves and lab coat • Label and markers

SN	Specimen Type	Collection Instructions	Required list of consumables
		4. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory	• VTM (for nucleic acid tests)
6.	**Respiratory specimens (Nasopharyngeal swab)**	1. Seat the patient comfortably, tilt the head back. Gently insert calcium alginate swab into posterior nasopharynx via nose. 2. Rotate swab slowly for 5 seconds to absorb secretions. 3. Remove the swab and place swab in transport medium (bacterial, viral as required). 4. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory	• Sterile swab with flexible shaft and soft tips • Transport Media (Stuart's / Amies transport media for bacterial culture) • Viral Transport media. (for nucleic acid tests) • PPE (Gloves, Mask, Gown, Eye protector) • Label and markers • Biohazard Bag • Disinfectant • Cold chain Box • Ice Gel pack
	Respiratory specimens (Oropharyngeal swab)	1.Hold the tongue down with tongue depressor. 2. Sample posterior pharynx, tonsils, and inflamed areas with a sterile swab. 3. Remove the swab and place swab in transport medium (bacterial, viral as required).	• Sterile swab with flexible shaft and soft tips • Tongue depressor • Transport Media (Stuart's / Amies transport media for bacterial culture) • Viral Transport media (for nucleic acid tests) • PPE (Gloves, Mask, Gown, Eye protector) • Label and markers

SN	Specimen Type	Collection Instructions	Required list of consumables
		4. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory	• Biohazard Bag • Disinfectant
	Sputum (expectorated)	1. Have patient rinse or gargle with water. 2. Instruct patients to take a deep breath and cough up sputum directly into a wide-mouth sterile container. Avoid saliva or postnasal discharge. Minimum volume should be about 1 ml. Collect into a sterile container. 3. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory.	• Sterile sputum container • Label and markers • PPE (Mask, lab coat and gloves) • Biohazard bag • Transport media (Optional like UTM) • Cold Chain Box • Ice gel packs
7.	**Urine -Midstream (Female & Male)**	1. Thoroughly clean urethral area (female) and glans (male) with soap and water. 2. Rinse area with wet gauze pads. 3. Hold labia apart (female) or retract foreskin (male), begin voiding. 4. After several milliliters have passed, collect 5-10ml of midstream portion without stopping flow of urine.	• Sterile, leak proof wide mouth container. • Label and marker • Gloves and Lab coat • Cold Chain Box • Ice gel packs

SN	Specimen Type	Collection Instructions	Required list of consumables
		5. Collect urine into a sterile wide mouther container. 6. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory 7. Transport to the laboratory within 2–3 hours of collection. If this is not possible**, do not freeze but keep the specimen refrigerated at 2-8°C.** Keeping the specimen refrigerated will decrease the risk of overgrowth of contaminating organisms.	
	Urine - (Indwelling Catheter)	1. Disinfect catheter collection port with 70% alcohol. 2. Use needle and syringe to aseptically collect 5-10 mL of urine. 3. Transfer specimen into a wide-mouth, sterile container. 4. Transfer urine into another collection container appropriate for testing. 5. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory. 6. Transport to the laboratory within 2–3 hours of collection. If this is not possible**, do not freeze but keep**	• Sterile wide mouth container • Sterile indwelling catheter • Drainage bag • Antiseptic solution (Chlorhexidine or iodine solution) • Gloves • Label and markers • Disposable bag

SN	Specimen Type	Collection Instructions	Required list of consumables
		the specimen refrigerated at 2-8 °C. Keeping the specimen refrigerated will decrease the risk of overgrowth of contaminating organisms.	
8.	**Skin- Wound swab**	1. Preferably collect specimen prior to initiation of therapy. 2. Avoid swab collection if aspirates or biopsy samples can be obtained. 3. Cleanse skin or mucosal surfaces. 4. For closed wounds and aspirates, disinfect as for a blood culture collection with chlorhexidine-alcohol or 70% alcohol followed by an iodine solution. Remove iodine with alcohol prior to specimen collection. 5. For open wounds, debride if appropriate, and thoroughly rinse with sterile saline prior to collection. 6. Swab viable infected tissue, rather than the superficial debris. 7. Gently roll the swab over the surface of the wound approximately five times, focusing on the area where there is evidence of pus or inflamed tissue. *Note:*	• Sterile Swab • Sterile leak proof container to hold the swab. • Sterile saline or transport media Such as UTM • Brain Heart Infusion broth for bacterial and fungal culture • Thioglycolate broth if anerobic suspected in deep wounds • Disinfectant (70% alcohol or chlorhexidine and iodine solution) • Gloves, mask and lab coat • Biohazard bag • Cold Chain Box • Ice gel pack

SN	Specimen Type	Collection Instructions	Required list of consumables
		Limit swab sampling to wounds that are clinically infected or those that are chronic and not healing. *Superficial or deep wounds, including bites, should be cultured only if there is purulence, chronic drainage or nonhealing.* *Burn wounds may not have organisms distributed evenly; sampling different areas of the burn is recommended.* *Blood cultures should be used to monitor patient status*	
	Skin- Lesion, Vesicle	1. Vesicular lesions, vesicles can be ruptured or ulcerate, ulcers that have crusted may not contain viable virus or viral antigens. 2. Samples are obtained by disrupting the surface of the lesion and collecting fluid with a swab or by aspiration with 26- or 27-gauge needle attached to a tuberculin syringe. 3. Specimens should be collected gently, without causing bleeding. 4. The base of the lesion should be scraped to collect cellular material for antigen detection. 5. Swab should be placed in VTM (for PCR) and transported.	• Sterile swab with Flexible shaft and a cotton or rayon tip • 26- or 27-gauge needle • Tuberculin syringe • Sterile leak proof container • Scalpel or lancet • Transport media (Stuart's/ Amies for bacterial culture) • VTM (For Nucleic acid tests) • Label and markers • PPE

SN	Specimen Type	Collection Instructions	Required list of consumables
		6. Transport to the laboratory at room temperature, store at refrigerate if delay in the transport and transport to the laboratory at 2-8 0C.	• Biohazard bag • Cold chain box • Ice gel Pack
	Crusting stage	1. Gently ease off crust with a lancet or scalpel and a pair of disposable forceps. 2. Take crusts from at least two sites; place them in a plastic screw-cap vial. Make sure the lid is tightly closed. Label the specimen containers.	• Scalpel or lancet • Forceps • Plastic screw cap vial • PPE • Label and marker
	Aspirates (Pus/Abscess)	**Should only be performed by trained personnel.** 1. Disinfect the skin overlying the abscess/bubo with 70% isopropyl alcohol Aspirate abscess wall material with needle and syringe. • Sampling of surface area can introduce colonizing bacterial not involved in infectious process. 2. Aseptically transfer all material into a sterile container. 3. Label container with two identifiers, source (site), date (time) of collection and transport with requisition to laboratory.	• Syringe and needle • Disinfectant (70% isopropyl alcohol) • Sterile leakproof container • PPE (gloves, mask, apron) • Label and marker • Biohazard bag

SN	Specimen Type	Collection Instructions	Required list of consumables
9.	**Urethral/Genital specimens**	1. *Genital ulcer swabs*, ulcers should be swabbed using dry swab and then placed into VTM (for PCR), or appropriate transport media (Stuart medium for bacterial culture). 2. Cervix specimens and cervical specimens are collected with swabs or brushes. 3. Prior collection mucus should be removed. 4. Endocervical specimens are collected by inserting swabs to a depth of 1 cm into the cervical canal and rotating it for 5 second. 5. Urethral/Cervical samples are placed in standard medium (bacterial/ viral transport medium based on test method).	• Sterile swab • Transport media (UTM or Stuart medium) • VTM • PPE (gloves, mask, apron) • Label and marker • Biohazard bag • Speculum • Torch light

ANNEX 2: COLLECTION AND TRANSPORT OF WATER SAMPLES FOR ISOLATION WATER-BORNE PATHOGENS

A. Clean [(Well, River (other clear water sources) Bottle/ Jar)] water

Materials required:

Pre-sterilized 500-ml screw capped glass or plastic bottle.

The bottles can be sterilized by autoclaving at 121°C for 15 minutes. The bottles can be reused after thorough washing but ensure no detergent or anti-bacterial residues are carried in the bottle.

Collection of water sample:

a. Uncap pre-sterilized plastic bottle. Fill to half of the volume of the bottle, re-cap, shake to rinse and discard. Repeat 3 to 5 times
b. Water from the tap should be collected only after running it from the tap for 2-3 minutes.
c. Water from streams or lakes should be collected in a bottle open only after immersed at a depth of 30 cm with its mouth facing the current.
d. Water from wells should be collected by bottles tied with heavy weight (stones).
e. Remove the sample bottle from water. Cap, leaving enough air in the bottle for agitation and mixing.

Transportation and/or processing:

Transport samples to the laboratory for processing with proper labelling as quickly as possible or at least within 6 hours or, preferably, begin processing onsite within 1 hr. of collection. Various biological and physiochemical factors, such as nutrient content, salinity, temperature, and pH, may influence the growth, survival, and distribution of V. cholerae in aquatic environments

For samples that require transport to the laboratory to be processed after collection, store and/or transport the samples in a cold box at 10° to 15°C. Alternatively, samples can be kept in the dark at ambient air temperature (22° to 25°C) after collection for up to 24 hours.

B. Environmental sample (sewage water/ muddy water)

Materials:

The Moore Swab.

Moore swabs (Pic. 2) are crafted using strips of cotton gauze cut into 6-inch by 48-inch lengths and folded eight times until an 8-ply square pad is formed.

The 6-inch by 6-inch square-pad is tied by a string, twine, wire, or fishing line around the center and sterilized in an autoclave.

Figure 5. *Moore Swab*

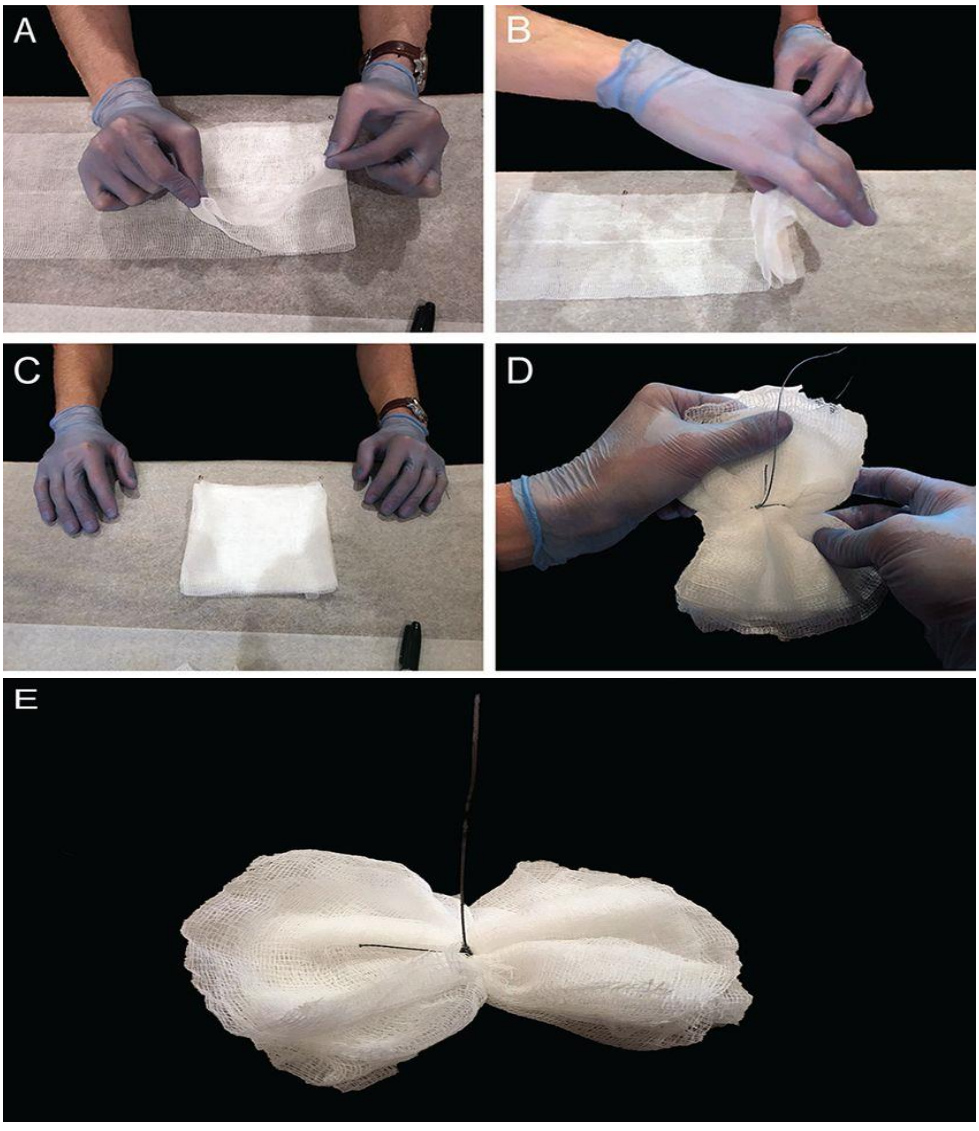

Figure 6. *Constructing a Moore swab. (A and B) A length of gauze, 6 inches by 48 inches, is folded onto itself in a pleated pattern to form a pad. (C and D) The gauze pad is tied at the center with high-test fishing line. (E) The Moore swab may be suspended in flowing sewers or surface waters.*

Sample collection method using moore swab.:

1. Sterilize Moore swabs by autoclaving prior to taking them to the field site.
2. Immerse the swab in the sewage or wastewater channel and tie it to a stable support using the tailing thread of the swab.
3. Leave in place for 24-72 hours
4. Prepare 450ml of Alkaline Peptone Water (APW) broth per sample in a glass bottle (Sterilized by autoclaving at 121oC for 15 minutes).
5. Cut the thread of the Moore swab and transfer the swab to an appropriately labelled container with 450ml of APW broth.

Transportation.

Transport the Moore swab APW as described above to the laboratory in room temperature protected from light within 24 hours.

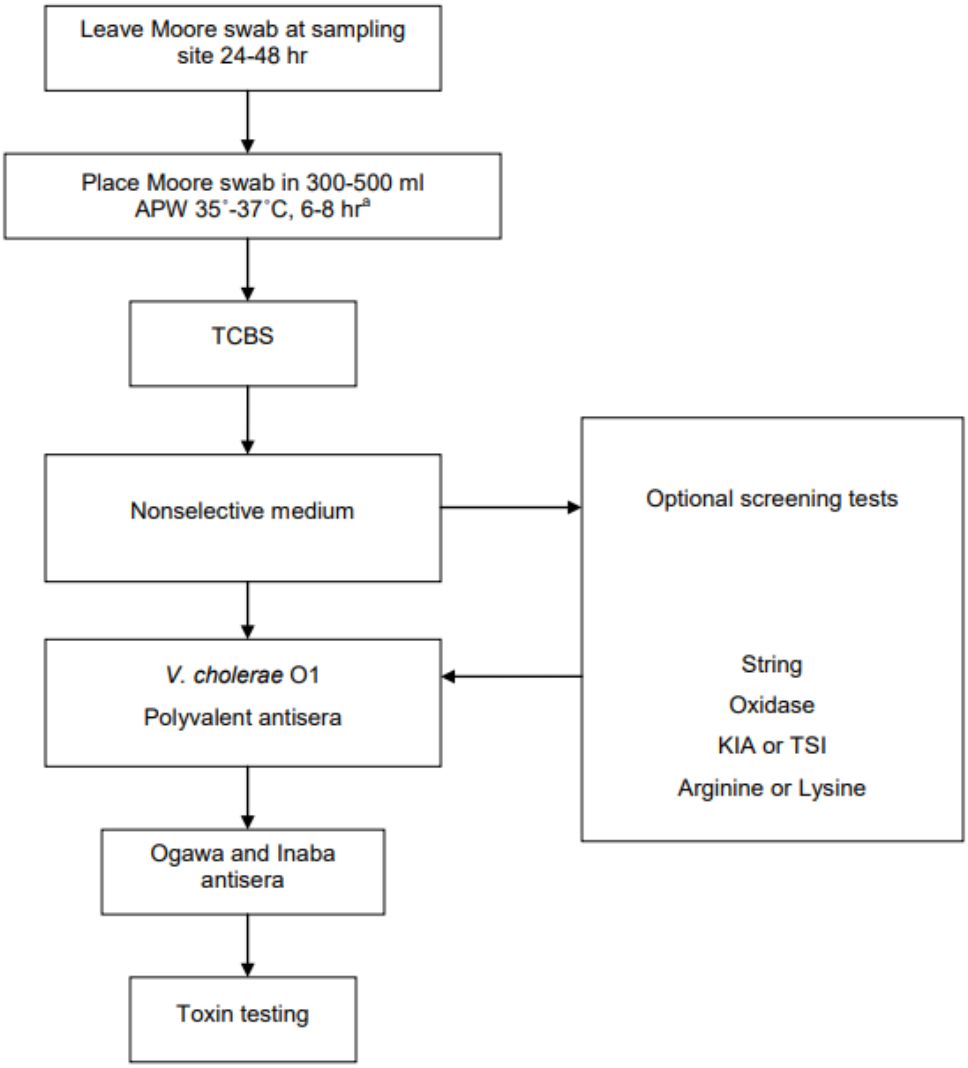

Figure 7. *Moore swab Technique for recovering of V. cholerae O1 from sewage water*

ANNEX 3: LABELLING FOR TRANSPORT OF BIOMEDICAL WASTES

Label for Transport of Bio-Medical Waste Containers/Bags	
Date	
Waste category	
Waste description	
Sender's Name, address & contact	
Receiver's Name, address & contact	
Emergency Contact Number	
Contact Name & Address	
Phone No.	

Bibliography:

Guidelines for the collection of clinical specimens during field investigation of outbreaks, World Health Organization Department of Communicable Disease Surveillance and Response, WHO/CDS/CSR/EDC/2000.4

Guidelines on Integrated Public Health laboratories,2021, Ministry of Health and Family Welfare Government of India

https://microbiologynote.com/types-of-blood-collection-tubes-and-their-uses/

Microbiology Essentials, Clinical Microbiology Specimen Collection Guidelines , Saskatoon Health Authority

Michael L. Wilson, General Principles of Specimen Collection and Transport, DIAGNOSTIC MICROBIOLOGY UPDATES, ern 1996;22

Laboratory Diagnosis of Viral Haemorrhagic, Fever Viruses, Viral Haemorrhagic Fever Guidance 2018 HPSC Guidance Document

Annex 2 of the International Health Regulations (2005) https://www.who.int/publications/m/item/annex-2-of-the-international-health-regulations-(2005)

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