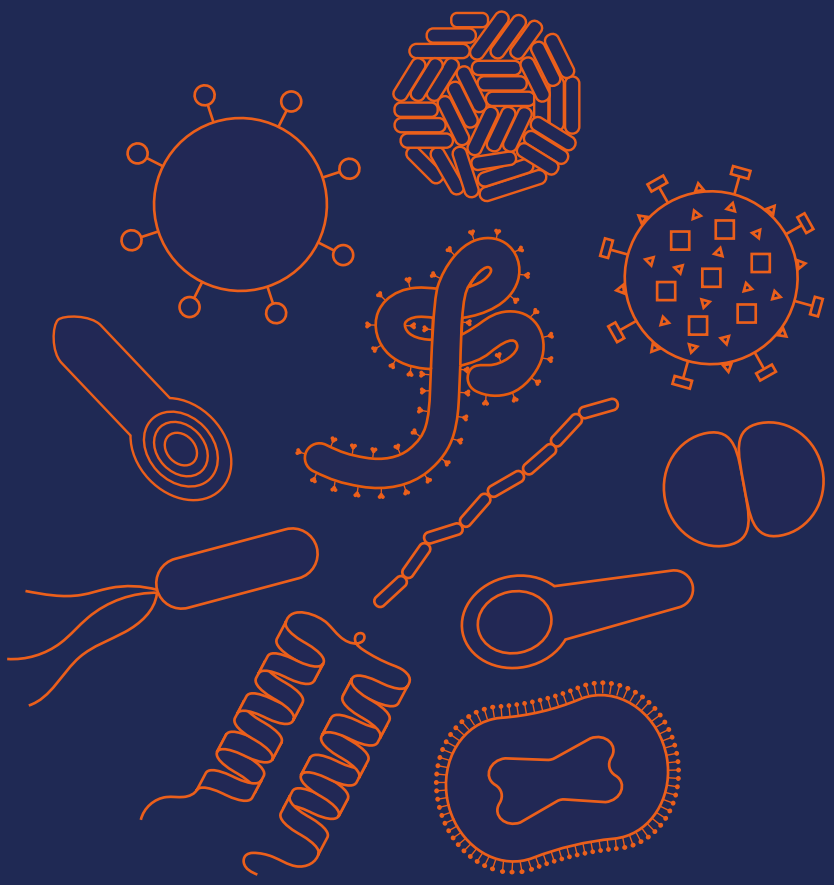
WHO global framework to define and guide studies into the origins of emerging and re-emerging pathogens with epidemic and pandemic potential



Developed by the
Scientific Advisory Group
for the Origins of Novel Pathogens (SAGO)

World Health Organization

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Contents

Executive Summary

There are a number of tools available for investigating infectious disease outbreaks, but there is not a unified, structured approach to investigating the origins of a novel pathogen's initial emergence or re-emergence. This World Health Organization (WHO) global framework - to define and guide studies into the origins of emerging and re-emerging pathogens of epidemic and pandemic potential - aims to fill that gap by providing a recommended set of investigations and studies to achieve this objective. It is the first version of a "how-to" guide that will be updated as and when needed.

The framework, developed by the Scientific Advisory Group for the Origins of Novel Pathogens (SAGO), complements other aspects of preparedness for the emergence of a novel pathogen. To conduct the investigations presented in this framework, countries should continue to build capacities necessary to conduct the investigations and studies herein. These are outlined in this document.

If the source of an outbreak pathogen can be identified early, it may be possible to halt chains of transmission before large-scale spread in the human population. If widespread human-to-human transmission is already occurring, identifying an animal reservoir, vectors or environmental source(s) may allow public health action to be taken to mitigate continued reintroduction into the human population. If the outbreak was a due to a laboratory biosafety or other biosecurity breach, improved measures can be put in place to ensure it does not re-occur.

To reduce risks, investigations into the origins must be started immediately after an outbreak is first detected and should be located, at least initially, in the place(s) where the first cases were detected. Relevant samples should be collected as soon as possible and submitted to appropriate laboratories with the capacity for pathogen discovery, isolation, genome sequencing and characterization to enable the development of diagnostic tests and gain insight into the evolution and transmission patterns of the pathogen.

A multidisciplinary investigation team should be assembled in the country where the pathogen/infection is first detected. Disciplines to be considered should include clinical medicine; epidemiology and public health; microbiology and virology diagnostics; infection prevention and control; laboratory biosafety; molecular biology; veterinary, wildlife, entomological, environmental, social, and anthropological sciences; bioinformatics; statistics; and data science. Subsequently, and as necessary, the country team can request assistance from international technical partners, such as WHO and the other members of the quadripartite: Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP) and the World Organisation of Animal Health (WOAH), and the Global Alert and Response Network (GOARN), to fill expertise gaps.

It should be noted that processes outlined in this framework are not linear or rigid. Investigations and studies may be conducted in parallel or in a different order than the one presented in this framework. Teams with appropriate expertise should work simultaneously on different sets of investigations and studies. For investigations and studies conducted, or being conducted, in relation to ongoing events that national authorities have notified to WHO, or verified, in accordance with the provisions of the International Health Regulations (2005), because of their potential to constitute public health emergencies of international concern, Member States should communicate regularly and share findings with WHO, as well as with local and national authorities, and other intergovernmental organization(s). At the same time, Member States should be linking to coordinated expert networks. Multiple reports of findings will be generated by these research teams, who will provide recommendations and make critical contributions to help guide next steps.

The recommended investigations primarily target novel pathogens of unknown origin. However, the investigations can also be applied to the re-emergence of pathogens with epidemic and pandemic potential (such as Ebola, influenza, Lassa fever, and mpox).

This framework outlines **six technical elements**, which provide examples of necessary investigations and studies that should be undertaken to determine the origins of a pathogen with epidemic or pandemic potential.

Technical Element 1: Early investigations aim to identify the earliest cases in an outbreak, the clinical and epidemiological features of these earliest cases, and to provide specimens for microbiological diagnosis and development and deployment of diagnostics to enable the subsequent elements of the investigation to be directed appropriately.

Technical Element 2: Human studies aim to investigate the clinical epidemiology of the disease in humans resulting from infection with the novel pathogen and how this can contribute to the understanding of its origin and populations at risk. This includes the occurrence of the disease in the early period of its recognition and any evidence for its occurrence in the period preceding the recognized start of the outbreak.

Technical Element 3: Animal-human interface studies seek to evaluate direct and indirect exposures from wild and domestic animals and explore transmission between potential ancestral hosts, intermediate hosts and humans, including identification of animal species susceptible to infection with the novel pathogen and the modes of how the virus entered the human population.

Technical Element 4: Environmental and ecological studies aim to evaluate the potential of environmental exposures to cause infection with a novel or re-emerging pathogen. This may be dynamic and involve the study of persistence of the pathogen on surfaces, food or water or in the air and relevant modes of transmission. These investigations are critical for implementing early preventive and control measures during an outbreak and may be used in retrospective studies to contribute to the timing and geolocalization of the origin of the epidemic.

Technical element 5: Genomic and phylogenetic studies provide viral or other pathogen genomics information to identify the pathogen involved, phylogenetic relation to the nearest ancestor and both spatial and temporal dynamics of pathogen circulation in humans and animals. It may also involve metagenomic analysis of environmental samples to identify any host genetic material that may link it to an animal or human host.

Technical Element 6: Laboratory biosafety and biosecurity studies aim to determine whether a disease outbreak is associated with a biosafety or biosecurity breach in a laboratory, during field research activities or in connection with another incident that may have led to occupational infection of workers or release into the environment and eventual spread into the local human or animal population.

In addition, the framework stresses the critical importance of **prompt and comprehensive reporting** of all aspects of origins investigations to all relevant national and international authorities.

Timely origins investigations with immediate attention to the site or sites where an infectious disease caused by a novel pathogen was first detected could potentially avert a massive public health crisis. As has been stressed throughout this Framework, success will hinge on two fundamentals: 1) close collaboration between appropriate government, public health agencies, research institutions and local partners in the country where the pathogen is first identified and openness to the support of international organizations such as WHO and

its technical advisory groups; and 2) full transparency regarding all findings so that immediate mitigation measures can be taken to prevent a pandemic.

Part I: Introducing the global framework

The COVID-19 pandemic has been a wake-up call for the world, demonstrating how a high-threat pathogen can cause massive global public health and socioeconomic impacts and disrupt all of society. It has also reminded the world of the importance of rapidly identifying a novel pathogen and determining how it could cause an outbreak.

It is essential to understand how a pathogen enters the human population, determine the risk, determine the risk of re-introduction and prevent future epidemics and pandemics. In the current context of an increasing rate of emergence or re-emergence of pathogens due to a number of factors, the task of stopping an outbreak in its tracks–before it spreads further–is more critical than ever.

A number of tools exist for investigating infectious disease outbreaks, but there is not a unified, structured approach to investigating the origins of a pathogen's emergence or re-emergence. This framework aims to fill that gap by providing a recommended set of investigations and studies to achieve this objective. It is the first version of a "how-to" guide using a One Health approach that will be updated as and when needed.

The framework complements other aspects of preparedness for the emergence of a novel pathogen. To conduct the investigations presented in this framework, countries should continue to build capacities (see Boxes 3-5) necessary to conduct the investigations and studies herein.

In 2024 the WHO R&D Blueprint for Epidemics updated a watchlist for pathogens that pose the greatest public health risk due to their epidemic potential. This list was subsequently updated to include COVID-19 (see Box 1), but it can by no means identify every candidate. At the time of publication of this framework, the R&D Blueprint team is updating this list of priority pathogens to include viral families that may give rise to emerging or re-emerging pathogens. The list includes "Disease X", acknowledging that there is an ongoing risk that an unknown pathogen will emerge, cause human disease and spark a future epidemic or pandemic. The WHO R&D Blueprint aims to improve cross-cutting R&D preparedness that is also relevant for a "Disease X". WHO has existing comprehensive programmes for influenza and other respiratory viruses, arboviruses and high-threat zoonotic viruses and epidemic bacteria, among others.

Box 1 – Diseases with epidemic potential prioritized by the WHO R&D Blueprint for research and development in emergency contexts by WHO (adapted 2018 list)*

- COVID-19
- Crimean-Congo haemorrhagic fever
- Ebola virus disease and Marburg virus disease
- Lassa fever
- Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome (SARS)
- Nipah and henipaviral diseases
- Rift Valley fever
- Zika
- "Disease X"

*This list has been updated in 2024 and organised by pathogen families, https://www.who.int/publications/m/item/pathogens-prioritization-a-scientific-framework-for-epidemic-and-pandemic-research-preparedness

Most high-threat pathogens that could cause epidemics and pandemics are zoonotic and have demonstrated the ability to spill over to humans (see Figure 1). They can then be amplified and spread between people in high-contact settings such as live animal markets or mass gathering events and in the context of increased human-animal contact, through arthropod vectors, global travel and/or international trade of animals. The risk of outbreaks is changing with increasing human incursion into animal habitats, increasing interactions with animals (in particular, wildlife), human population growth, intensive animal production and a rise in the number of animal markets and consumption of animal foods, as well as climate change. All increase the risk for pathogen spillover and amplification.

If the source of the outbreak can be identified early, it may be possible to interrupt the chain of transmission before large-scale spread in the human population. If widespread human-to-human transmission is already underway, identifying the possible animal reservoir, vectors or environmental source(s) may allow public health action to be taken to mitigate continued re-introduction into the human population. If the outbreak is due to a biosafety or biosecurity breach in laboratories or in the context of other high-risk activities such as field or medical research or in agricultural settings, improved measures can be put in place to ensure it does not have the chance to re-occur.

To reduce risks, investigations into the origins must be started immediately after an outbreak is first detected and should be focussed, at least initially, on the places where the first cases were detected. Relevant samples should be collected as soon as possible and submitted to laboratories with the capacity for pathogen discovery, isolation, genomic sequencing and characterization to enable the development of diagnostic tests and gain insight into the evolution and transmission patterns of the pathogen.

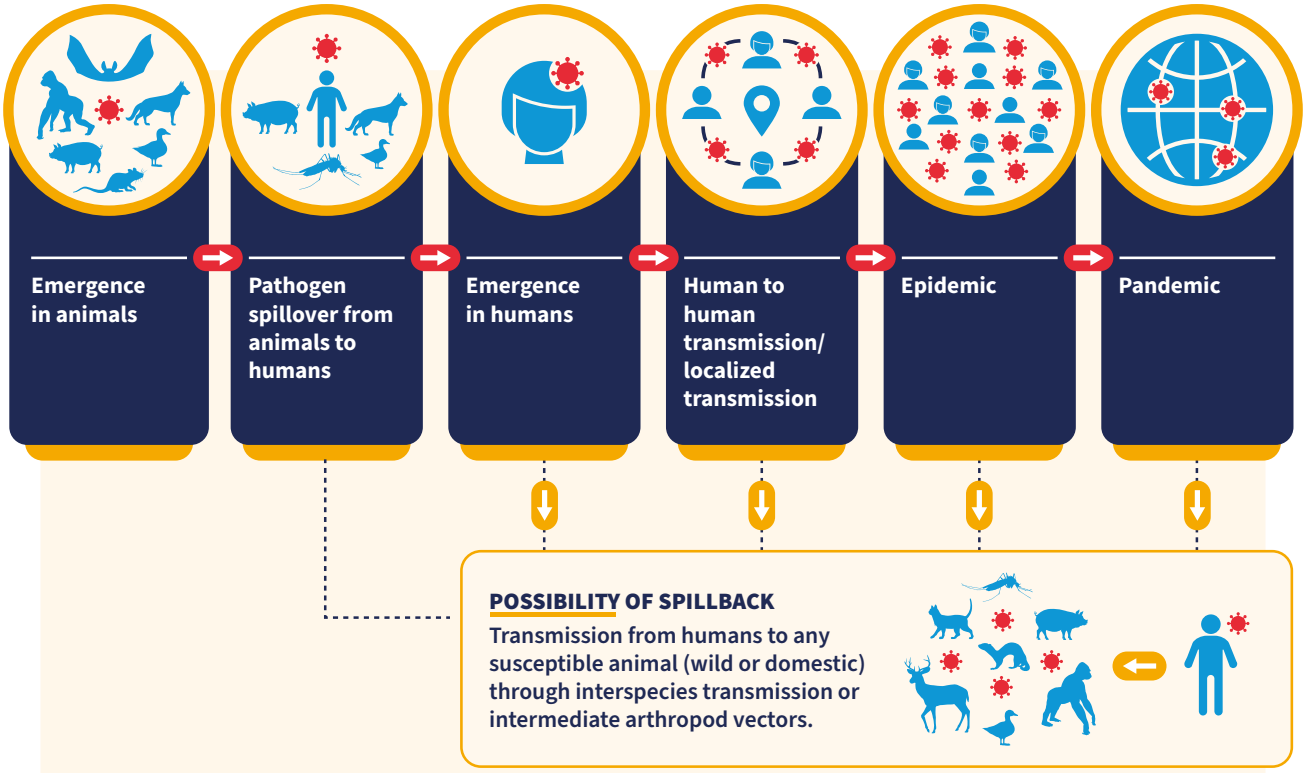
The framework includes recommendations on sets of comprehensive research studies and scientific investigations to obtain an understanding of the mechanisms of the entry of a novel pathogen into the human population and the dynamics of the early pathogen transmission and spread, including determinants that might have precipitated the emergence of the pathogen. It seeks to assemble the different methods, tools and strategies using a One Health approach to standardize the investigations and their immediate reporting. Finally, it calls for a participatory and integrative approach by considering diverse specializations and expertise essential for executing the various elements. It is not intended to be a comprehensive compendium of all the measures that need to be instituted to investigate a novel pathogen. Rather, it should be read in conjunction with other guides on mitigation of emerging diseases and new information about a disease as it becomes available. It can thus serve as a basis for national and local authorities to develop their own procedures and tailor them to their specific needs.

It should be noted that processes outlined in this framework are not linear or rigid. Investigations and studies may be conducted in parallel or in a different order than the one presented in this framework. Teams with appropriate expertise should work simultaneously on different sets of investigations and studies. For investigations and studies conducted, or being conducted, in relation to ongoing events that national authorities have notified to WHO, or verified, in accordance with the provisions of the International Health Regulations (2005), because of their potential to constitute public health emergencies of international concern, Member States should communicate regularly and share findings with WHO, as well as with local and national authorities, and other intergovernmental organization(s). At the same time, Member States should be linking to coordinated expert networks. Multiple reports of findings will be generated by these research teams, who will provide recommendations and make critical contributions to help guide next steps.

The recommended investigations primarily target novel pathogens of unknown origin. However, the investigations can also be applied to the re-emergence of pathogens with epidemic and pandemic potential (such as Ebola, influenza, Lassa fever, and mpox). Table 1 in Annex 2 developed by the SAGO, includes a non-

exhaustive list of possible emerging or re-emerging pathogens and includes viral and bacterial families of concern with examples of members of each family. This table should be consulted in conjunction with the updated R&D blueprint priority pathogen list.

Figure 1: Stages of emergence, spillover and potential spillback through zoonotic spillover



Development of the global framework

This document was developed through the efforts and collaboration of the Scientific Advisory Group on the Origins of Novel Pathogens (SAGO), a scientific advisory group to WHO that was formed in November 2021. The group is composed of experts acting in an individual capacity and serves to advise WHO on technical and scientific considerations regarding how best to investigate the origins of emerging and re-emerging pathogens (for more information about the SAGO, see Annex 1).

The framework was developed for use by scientists, researchers, public health authorities, and investigators with input from technical experts from specialist areas such as the animal-human interface, laboratory biosafety, clinical management, food safety, environmental science, laboratory science, occupational and public and animal health, epidemiology and pathogen genomics and others. It incorporates practical field experience gained by investigators working at international, national and subnational levels during investigations of outbreaks and epidemics, as well as incorporating experiences and lessons learned from previous investigations of outbreaks caused by pathogens including SARS-CoV-1, SARS-CoV-2, avian influenza (e.g. H5N1), Lassa fever, Chikungunya, Zika, Ebola virus disease, MERS-CoV, and mpox, among others.

Elements of the framework: overview

This framework outlines six technical elements – which outline necessary investigations and studies critical to determining the origins of a pathogen with epidemic or pandemic potential. The early investigation element must come first, since it is triggered by an outbreak of human illness of unknown origin, but as noted earlier, the other five elements need not be performed through a linear process. The studies should be complementary and conducted with consideration of the pathogen. Examples of existing tools that can be used across all stages of a pathogen's origin investigations can be found in the tools section in Annex 4. The six technical elements are summarized below and are presented in greater detail in Part 2 of this framework.

Technical Element 1: Early investigations aim to identify the earliest cases in an outbreak, the clinical and epidemiological features of these earliest cases, and to provide specimens for microbiological diagnosis and development and deployment of diagnostics to enable the subsequent elements of the investigation to be directed appropriately.

Technical Element 2: Human studies aim to investigate the clinical epidemiology of the disease in humans resulting from infection with the novel pathogen and how it can contribute to the understanding of the origin of a novel pathogen and populations at risk. This includes the occurrence of the disease in the early period of its recognition and any evidence for its occurrence in the period preceding the recognized start of the outbreak.

Technical Element 3: Animal-human interface studies seek to evaluate direct and indirect exposures from wild and domestic animals and explore transmission between potential ancestral hosts, intermediate hosts and humans, including identification of animal species susceptible to infection with the novel pathogen and the modes of how the virus enters the human population.

Technical Element 4: Environmental and ecological studies aim to evaluate the potential of environmental exposures to cause disease with a novel or re-emerging pathogen. This may be dynamic and involve the study of persistence of pathogen on surfaces or in water, food or air and relevant modes of transmission. These investigations are critical for implementing early preventive and control measures during an outbreak and may be used in retrospective studies to contribute to the timing and geolocalization of the origin of epidemic.

Technical element 5: Genomic and phylogenetic studies provide viral or other pathogen genomics information to identify the pathogen involved, phylogenetic relation to the nearest ancestor and both spatial and temporal dynamics of pathogen circulation in humans and animals. Once the pathogen genetic sequence has been obtained, it is compared through phylogenetic analysis to other closely related sequences on public databases to identify the closest related sequences that may be the nearest ancestor and provide information on the potential reservoir or intermediate host, estimated period since spillover and geographical location of the closest related viruses. It may also involve metagenomic analysis of environmental samples to identify any host genetic material that may link it to an animal or human host.

Technical Element 6: Laboratory biosafety and biosecurity studies aim to determine whether a disease outbreak is associated with a biosafety or biosecurity breach in a laboratory, during field research activities or in connection with another incident that may have led to occupational infection of workers or release into the environment and eventual spread into the local human or animal population.

Reporting, integration of findings and next steps

The comprehensive reporting of all aspects of origins investigations via integration of evidence generated across the technical elements of this framework is critical to inform other studies that will be needed. Reporting of any findings to relevant national and international stakeholders should occur promptly as results become available and involve the evaluation of the comprehensiveness and compatibility of assessments to guide discussion, control measures and next steps of the scientific investigations. This also entails providing a more holistic understanding of the investigated event, pathogen, and related implications. Reporting and consolidating findings will allow for comprehensive risk assessments of pathogen re-emergence in the affected country or other parts of the world and the planning of relevant prevention and control measures.

Triggers to implement the framework and activate an investigation into the origins of a novel pathogen

Unexpected signals from surveillance systems that suggest an unusual event require verification to identify true threats or in some cases, false alarms. If verified, they should trigger an immediate response by relevant authorities who should also consider activation of this framework. Some examples of triggers include but are not limited to the following:

- **A cluster or clusters of acute infections** in humans of unknown origin with moderate to severe clinical signs. This may include febrile disease with or without respiratory, neurological or haemorrhagic fever signs or other symptoms that result in an increase in health care-seeking behaviour, hospitalization or unexplained deaths among a group of people from a geographic area or with another epidemiological link *(1)*.
- **A possible link** between infections and recent exposures to animals or animal products, insect bites or environmental conditions such as contaminated water sources, food or live animal markets or occupations that may present an increased risk of infection.
- **Infections associated** with moderate to severe clinical signs in a population of health workers who care for patients with similar unexplained acute clinical signs likely caused by an unknown infectious agent. Syndromes of concern may include respiratory infections, haemorrhagic fever signs, neurological manifestations, hepatitis, or any febrile disease of unknown origin.
- **Acute neurologic manifestations** in a given population with recent exposures to infected animals or vectors, especially in new regions (e.g. arboviruses such as Japanese encephalitis, West Nile encephalitis, Zika).

- **Abrupt, unexplained changes** in the trends of disease occurrence or clinical outcomes from known pathogens, including deaths observed in local health systems and/or in routine surveillance activities.
- **Re-emergence of known pathogens with epidemic and pandemic potential.** These include blood-borne pathogens such as haemorrhagic fever viruses (e.g. Ebola virus, Marburg virus), poxviruses such as mpox virus, smallpox (variola) viruses or other zoonotic respiratory viruses such as Nipah virus, SARS-CoV, MERS-CoV or novel variants of concern of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); zoonotic or unsubtypeable influenza and other zoonotic respiratory viruses that spilled over to humans; zoonotic bacterial pathogens with epidemic potential such as Yersinia pestis (pneumonic/bubonic plague); the emergence of epidemic-prone and zoonotic arboviruses in new regions, re-emergence of wildtype poliovirus, or known pathogens with increased virulence, among others (see Annex 2).

Events should be reported to national or international public health authorities in accordance with the International Health Regulations (IHR) (2005) *(2)*. The investigations recommended in this framework should be initiated and run concurrently with the country's emergency outbreak response plan. WHO has created outbreak toolkits for various diseases.

Who should conduct the origin investigations

A multidisciplinary investigation team should be assembled in the country where the pathogen/infection is first detected. Disciplines to be considered for the multidisciplinary investigation team should include clinical medicine, epidemiology and public health, diagnostic and reference microbiology, infection prevention and control, laboratory biosafety, molecular biology, veterinary, wildlife and entomological science, environmental science, social science and anthropology, bioinformatics, statistics and data science; along with finance, logistics and political support (Box 2). Subsequently, and as necessary, the country team can request assistance from international technical partners, such as WHO and the other members of the Quadripartite: Food and Agriculture Organization of the United Nations (FAO), United Nations Environment Programme (UNEP) and the World Organisation of Animal Health (WOAH), and the Global Alert and Response Network (GOARN), to fill expertise gaps.

Box 2. Resources needed to implement the framework

Multi-disciplinary team
A multi-disciplinary team with relevant technical expertise. The team should include individuals with familiarity with the local context and individuals with links to national and international expertise and resources.

Finance and logistics
Financial and logistical support to the team and others carrying out related investigations. This should include the capacity for sample and data collection, analysis, storing and sharing.

Political support
Local and national governmental and international political support for the investigation from WHO and the other Quadripartite organizations and other intergovernmental authorities.

Laboratory capacity
Access to local, national and international laboratories for the development and deployment of diagnostics, reference capacity and research.

Field investigations
Safe access to relevant field sites and foci of early cases and experienced field investigators including medical, veterinary and epidemiologic or environmental scientists.

Health data
Access to local and national health data including relevant health service utilization, and routine surveillance data from the country's official public health and private health authorities.

Other relevant data
Access to local and national data on activities that may be relevant to the outbreak development including transport, trade, events, and cultural practices.

Capacities and infrastructure required to conduct origins investigations

While some countries have the personnel, capacities and infrastructure in place to conduct the origins investigations described in this framework, others may need to establish partnerships with other countries or international entities. WHO and the other members of the Quadripartite (FAO, UNEP, and WOAH) and the Global Alert and Response Network (GOARN), can provide support as needed. WHO urges all countries to begin or continue taking the necessary steps to establish the critical capacities (Box 3), laboratory expertise (Box 4) and data collection capacities (Box 5) outlined below.

Box 3. Critical capacities to support origin investigations

- A multi-sectoral plan to support an operational response including a national or international multidisciplinary team of scientists available for rapid assembly to begin investigations
- Ability and willingness to collaborate with WHO and the GOARN to support local expertise, as needed
- Surveillance systems to identify unusual data trends, such as in influenza-like illness and severe acute respiratory infection and acute flaccid paralysis or neurological infection; wastewater surveillance for enteric diseases/polio; zoonotic disease or arbovirus surveillance in animals and humans
- Secured database platforms to ensure the collection and completeness of good quality and accessible epidemiological data on human cases, animals, environmental components, genomics and laboratory samples
- Collection and storage from surveillance programmes of routine samples (human, animal and environmental) available for pathogen testing and sequencing (including pathogen isolation and molecular characterization and serological investigations)
- Capacity to compare results from different laboratories in accordance with international reference standards
- An infection and prevention control monitoring plan for health facilities.

Box 4. Laboratory characteristics and capacities to support origin investigations

- Agility to increase diagnostic testing efforts and capacities in times of an outbreak investigation
- Capacity for virus and bacterial collection, isolation, multi-pathogen molecular diagnostics assays including specific real-time polymerase chain reaction (PCR) or for known pathogens and genus PCRs for a range of families or multi-pathogen arrays assays as well as Sanger and next-generation sequencing capacity for pathogen discovery (reference and/or research laboratories)
- Prepared for rapid and specific pathogen identification (e.g. diagnosis, isolation and genomic sequencing) to generate data needed to better understand the event and inform actions and the overall response
- Conforming to laboratory biosafety and biosecurity regulations, with governance and oversight in place requiring they provide data on their management in the event of an outbreak
- For those working with biological agents, accurate and thorough documentation to allow for easy tracing of types of pathogens studied, types of laboratory biosafety/biosecurity protocols in place and occupational health records in case of any adverse events
- Ability to collaborate and form agreements with WHO to confirm the validity of positive early clinical samples, virus isolates, and sequence findings at a reference laboratory involving the WHO lab systems; the WHO BioHub System (which was being piloted at the time of this writing and can offer a reliable, safe, and transparent mechanism for WHO Member States to voluntarily share novel biological materials without replacing or competing with existing systems); or one of the WHO collaborating centres.

Box 5. Recommended data collection systems and capacities (by technical element)

Early investigations

This involves outbreak investigations and includes collection of clinical human, animal or environmental samples, intelligence data, epidemiological information at the time and identification of index cases.

Capacity to identify the following is required:

- Earliest recognized cases including clinical and demographic features, and activity in the period prior to the onset of symptoms in index cases
- Potential chains of transmission
- Places visited in a defined period prior to the onset of symptoms
- Identified exposures to animals (or animal products) or other potential sources of infection.

Human studies element

Capacity to identify or gather the following:

- Collated data on early cases, including those identified as part of the initial outbreak, and subsequent secondary cases
- Case definitions and methods of ascertainment used to identify the early cases
- Data from local health services about the occurrence of relevant illness in the population in both the early period of the outbreak and the period preceding the recognition of the outbreak
- Data from routine local, regional and national surveillance systems for relevant syndromes in the population in the period prior to the recognition of the outbreak (should include syndromic as well as disease specific data, and mortality data)
- Pharmacy data for sales/prescriptions for the management of relevant symptoms in the period preceding the recognition of the outbreak
- Data on events and other human behaviours in the local population that may point to potential links to a relevant exposure.

Animal-human interface element

- Veterinary surveillance systems for livestock, wildlife and domestic animal diseases; veterinary public health surveillance of animals at markets, animals hunted for bushmeat, abattoirs or livestock from farms that supply these facilities and/or domestic animals with human contact at sites where the source of zoonotic spillover could be identified
- Surveillance of potential reservoir species of zoonotic diseases (e.g. bats, rodents, wildlife, wild birds to detect closely related precursor strains through genus-specific molecular testing or sequencing) and serological testing
- Secure platforms to ensure the collection and completeness of good quality and accessible epidemiological and laboratory sample data
- An established system for One Health collaboration across human, animal and environmental health sectors should be in place. This group should support surveillance and laboratory systems and reporting of cases in animals and include social scientists and anthropologists to identify risk factors for spillover and tracing of the origins of the novel pathogen.
- Bio-banked samples from domestic animals or wildlife to identify progenitors or ancestors of a novel pathogen.

Environmental and ecological studies element

- Wastewater and environmental sampling that will allow metagenomic investigations to identify the presence of a pathogen and potential human or animal hosts present at the time of an outbreak.
- Systematic sample collection, analysis and preservation of environmental samples to support surveillance.
- Arthropod vector surveillance, species abundance and distribution maps and interaction with the human population and vector susceptibility for known and novel vector-borne pathogens.

Genomics and phylogenetics element

- Local databases for the pathogen genome sequencing data should be established and shared with either GISAID, GenBank or other open-source databases, together with metadata that include geographic, demographic, clinical and epidemiological data, as well as potential animal hosts
- Secured database platforms in place to ensure the collection and completeness of good quality and accessible epidemiological data on animal syndromes and diseases, genomics and laboratory samples. Raw sequencing data for quality control should also be available and stored safely for traceback activities.

Laboratory biosafety and biosecurity element

- Laboratories should have capacity to maintain documentation and records of their practices and make them available to investigative teams according to the WHO Laboratory biosafety manual, 4th edition. Information on laboratories working with novel pathogens that have re-emerged or are extinct or any other epidemic-prone pathogen, should be accessible as should details about the activities carried out within each facility or where field studies are conducted, including the biosafety and biosecurity systems in place (e.g. occupational health records).

Part 2: Technical elements and recommended investigations

Early investigations

Element scope

Early investigations aim to identify the earliest cases in an outbreak, the clinical and epidemiological features of these earliest cases, and to provide specimens for microbiological diagnosis and development and deployment of diagnostics to enable the subsequent elements of the investigation to be directed appropriately. Some aspects may overlap with the other five targeted elements described in this framework.

These investigations include preliminary studies, scientific investigations, situational assessments, and collection of samples for analysis later in the outbreak investigation and response. Collection of samples during the early phase of any outbreak is highly time sensitive and should follow the principle that "rather too many than too few". If not analysed immediately, samples can be stored properly for subsequent laboratory analysis. Sample collection should take place even if the country where the outbreak occurs lacks laboratory capacity to conduct all required analyses. If so, the investigative team can connect with laboratory networks in other countries able to provide this function. WHO can support additional technical support for sharing of specimens for detailed characterization.

Annex 3 outlines a list of recommended samples and materials to be taken during the early stages of scientific investigations.

Key areas of investigation

- Epidemiologic investigations
- Site visits, sampling of human cases and animals and environmental samples for identification and characterization of the pathogen involved
- Genomic sequencing of specimens from early cases and development of diagnostic tests
- Determination of modes of transmission and the extent of human-to-human and/or animal-to-human transmission
- Identification of any nosocomial transmission in healthcare centres
- Occupational health records of any laboratory biosafety or biosecurity incidents related to research activities in laboratories or in the field
- Index case investigations to capture possible occupational exposures.

Early investigations are critical for understanding of the origin of a pathogen at the time of its first emergence. They can provide a crucial understanding of the possible animal hosts or reservoirs associated with the earliest cases and the associated human behaviours and practices that increase the odds of acquiring the infection.

Part of the investigation involves mapping needed human and material resources and defining an initial hypothesis and key investigation questions and priorities. The investigation team should be a national multidisciplinary One Health team that could be complemented and supported by an international team coordinated by WHO and other partners such as WOAH and FAO. Good preparations and an action plan denoting roles and responsibilities are essential for the success of the early investigation, which can be divided into two broad categories: scientific and investigative issues and management and operational issues.

Following the verification of an outbreak – including reviewing the number of cases and deaths – all relevant fields, administrative and ethical approvals should be obtained in accordance with national laws. Priority should be given to monitoring studies with a One Health approach: collecting biological samples among humans, wildlife and domestic animals and potential animal or arthropod vectors. These investigations can assess the feasibility of implementing the five targeted elements of this framework and determine the factors for either the success or the failure of performing further targeted studies. Coordination of investigations and sharing of information in real time will be needed at both country and global levels.

Several tools may be useful for performing outbreak investigations; including the WHO Unity Protocols to investigate non-seasonal influenza and other acute respiratory diseases, the Middle East Respiratory Syndrome Outbreak Toolbox or the national protocols, WHO's Standardized data collection tools and others established by national authorities.

Recommended studies

- Clinical assessments should be carried out to describe the typical, and range of, clinical presentation to support the early development of a clinical case definition. This definition should include clear descriptions of the range of severity and clinical manifestations, including different human clinical features, animal cases and the range from asymptomatic to symptomatic infection. These data will enable investigators to properly evaluate potential retrospective cases. The early case definition should be reviewed and updated and amended as needed.
- Case descriptions should include syndrome descriptors, patient characteristics–such as age, sex, pre-existing conditions, known exposures (if this knowledge is available)–and possible spatiotemporal medical restrictions that define probable exposures.
    - Immediate epidemiological investigations should aim to identify the extent of infections and clarify modes of transmission and the extent of ongoing human-to-human transmission, with possible information on past or ongoing animal-to-human transmission and potential environment-to-human-transmission.
    - Hospital epidemiological investigations should examine exposures and nosocomial infections in health workers occurring in health care or related settings.
- As noted earlier, sampling should be carried out to ensure samples are available for immediate investigation and studies (see the WHO laboratory safety manual). Required samples are determined by the syndrome and include relevant bodily fluids, where possible. The minimum sample set in all cases is whole blood, serum, and nasopharyngeal and oropharyngeal swab specimens[1]. These should be safely stored to allow for investigations to be carried out later. Depending on the syndrome, there may also be a need for lower respiratory tract specimens from hospitalized patients with pneumonia; cerebrospinal fluid for neurological syndromes; scabs/lesions for skin conditions; or post-mortem samples, which should include visceral organs; lungs, liver, spleen, lymph nodes and central nervous system (brain and spinal cord, if neurological).
- Site visits of the locations (e.g. markets, farms, gatherings) where the first detected cases have spent time should be conducted for site documentation and involve interviews with all involved persons.

1. To note, as the outbreak unfolds, WHO will publish up-to-date evidence-based recommendations for safe collection of biological materials.

This should include registration of absent persons for later interviews, anamnestic interviews to reconstruct unreported exposures and complex exposure histories, sampling in persons with potential unreported exposures (e.g. swabs and serum) and informing groups of potentially exposed people regarding general measures of infection control adapted to the situation. Any disturbance of sites where early cases were detected, including cleaning, should be assessed and interrupted, if appropriate.

- All investigations and situation assessments should ensure early specimen collection and availability for biobanking (humans, animals, environment) samples.
    - **Humans**: Biological material from early human cases and persons sampled during site visits should include appropriate clinical samples including sputum, nasopharyngeal or oropharyngeal swabs, blood, cerebrospinal fluid, urine, stool or rectal swabs and semen samples, depending on the syndrome. If possible, pathology and/or autopsy samples should be sought. The minimal samples set should include full blood, serum and nasopharyngeal swabs samples.
    - **Animals and vectors**: In cases with suspected exposures to domestic or wild animals and related upstream supply chain, biological materials needed will include samples from animals and animal products as well as from environments where they were kept. The minimum sample set should be full blood, serum, faeces, urine, milk, oropharyngeal or nasopharyngeal, faecal/anal swabs and visceral organs (lung, spleen, liver, heart, kidneys) or brain and spinal cord for neurological cases following necropsy or slaughtering, if applicable. In the case of suspected or non-excluded vector-borne aetiology, an arthropod inventory sample consisting of mosquitos, ticks, sandflies, Culicoides biting midges, flies and other abundant arthropods should be taken.
    - **Environment**: In cases with suspected or non-excluded environmental exposure, early investigation sampling should include environmental surfaces, drainages, animal cages and wastewater from suspected sites of exposure (samples from food products and water). In the case of artificial ventilation, air conditioning or technical cooling, air and air filter sampling should be considered.
- Laboratory investigations of samples from the index cases of an outbreak of a specific syndrome of unknown cause that could not be identified by routine testing should be submitted at a reference or research laboratory with the capacity to do virus/bacterial isolation at BSL2 or higher biosafety level, depending on the syndrome. This may be done in the country, if it has the laboratory capacity, or sent to one of the WHO collaborating centres. Additional laboratory analyses include multipathogen molecular investigations using genus PCRs, micro-array systems, or NGS-sequencing or electron microscopy to determine what category of pathogen is involved. Antigen detection with broadly reactive antibodies or serology and serum neutralization assays may also be used to determine exposure to different pathogens. This will also allow for quick development of a rapid diagnostic assay for more targeted investigations.
- Samples from humans with suspected exposure should be collected and preserved for the investigation as part of surveillance programmes. Samples to be characterized include stored blood and serum tubes from clinical chemistry and haematology processes; lookback samples from blood banks (examined to ensure there were no laboratory errors during testing); and original and sub-processed samples from microbiology, virology and serology testing and nucleic acids extracts from molecular biology testing (genetic and microbiology/virology). If possible, samples should be stored at -80°C and transported to long-term storage in liquid nitrogen or dry ice containers. Long-term storage should be planned in accordance with local and national guidance.

Human studies element

Element Scope

Human studies aim to investigate the clinical epidemiology of the disease in humans resulting from infection with the novel pathogen and how this can contribute to the understanding of its origin. This includes the occurrence of the disease in the early period of its recognition and any evidence for its occurrence in the period preceding the recognized start of the outbreak. They should be linked to investigations of the pathogen in animals (and animal products) and the environment.

The earliest recognized cases are likely to have symptoms and possibly severe illness because these patients typically seek medical care (the earliest signals of a new illness are often an increased number of hospitalizations or deaths) and are often detected first by national surveillance systems. The signs and symptoms of the illness provide the basis for an initial case definition which, in turn, is valuable for identifying the emergence of the disease in different population groups and geographic areas.

Investigation into early cases also provides a focussed opportunity to collect relevant specimen materials to examine the microbiological factors associated with the disease and its transmission and to evaluate and validate diagnostic approaches to identify the disease. Typically, however, there will be other cases of infection with milder illness or cases without any apparent symptoms, who are not seeking healthcare and they form an equally important component of understanding the epidemiology of the infection. Such cases must be detected using methods recommended in the early investigations element to better understand the modes of transmission. The development of a robust set of laboratory diagnostics (serologic and direct microbe testing) may be critical to characterize the spectrum of illness (see, for example, the WHO Unity Studies protocols).

Human behaviour plays an important role in understanding the origins of a novel pathogen by tracing back its pathway into the human population and its subsequent spread to other humans, animals and the environment. Relevant human behaviour may be affected by a wide range of cultural, social, educational, economic, trade, transport, health, nutritional, geographic, and climatic factors.

Key areas of investigation

- Use all available clinical and epidemiological information from the first recognized cases that may indicate time, place and person aspects of initial exposures and transmission pathways into the human population and spread from human to human or other sources of sustained infection.
- Combine the information from clinical epidemiological data and diagnostics with molecular sequencing data from the earliest cases to better understand their relatedness and common ancestry.
- Use local syndromic surveillance and other sources of information (such as the number of hospitalizations and burials) and samples to search for other unrecognized early cases that might contribute additional early epidemiological information, such as potential nosocomial outbreaks.
- Use surveillance and other sources of information and samples from local, regional and national procedures to search for evidence of earlier (than reported) potential/suspected human infections and transmission due to analysis of the novel/re-emerging pathogen.
- Combine information from human studies with information from studies of the animal-human interface and environmental studies together with information about relevant human behaviours.

Recommended studies

The quality of the data used in each of the following investigations should be evaluated regularly to assess the reliability of conclusions drawn and to inform next steps.

- Evaluate the symptoms, signs and severity of early repeated human cases and the extent of known infection to gain an understanding of the range of clinical illness.
- Collect detailed information from initial cases to develop a standardized approach to evaluating subsequent confirmed, probable, suspected cases and ultimately serve to develop case definitions. If new information becomes available that was not recognized at the time of ascertainment of the initial cases, re-interviewing initial cases should be considered. This investigation may include determining the strength of the association between initial cases and proposed risk factors based on the findings from the early descriptive epidemiology or other factors such as local events, occupational hazards (for example, among health workers), live animal markets, interaction with animals and the environment and other human behaviours.
- Carry out descriptive epidemiological analysis (time, place and person) of the initially identified cases, informed by case definitions and diagnostic methods to obtain an early indication of when, where, how and in whom the new disease first arose and potential modes of transmission.
    - Continuously review and revise case definitions in the light of updated information about the disease. Early case definitions will generally be highly specific and reflect the initial recognition of clinically severe cases. Subsequent definitions should be more sensitive, including the identification of milder disease or asymptomatic infections.
    - The methods used to identify cases within the affected population will influence the representativeness of the cases in the initial analyses and should be carefully considered and recorded. Subsequent approaches to case ascertainment may need to be revised in the light of updated information about the occurrence of the disease.
- Develop and validate (or use available) sensitive specific molecular diagnostic assays (real-time PCR) after the initial pathogen identification. They can be used for rapid identification of additional cases and sent to other countries to detect wider spread.
- Develop and validate (or use available) serological assays to identify evidence of recent or past infection with the novel pathogen.
- Conduct enhanced surveillance such as serosurveys, which can provide information about the prevalence of infection, incidence of cases and factors associated with, new infections in combination with clinical and epidemiological information on those tested (see, for example, the WHO Unity Studies protocols). Relevant specimens should be sought from the period immediately preceding the onset of the first cases and at intervals following the onset of the outbreak, with the interval timing being determined by the epidemiological characteristics of the infection. Collections of samples from the months and years before the start of the outbreak should also be sought to provide historical comparisons.
- Apply anthropological, social science and behavioural approaches to determine relevant aspects of exposure in the affected population in the period preceding and during the recognized emergence of the epidemic that could have contributed to its emergence in humans, amplification of early human infections and/or subsequent onward spread in the population.
- If applicable, review ‘event-based surveillance’ to determine if there may have been community

events that could have amplified the spread of the disease. These may include mass gatherings, major festivals, sporting and other entertainment events and holidays. Information from articles, publications or other records (official and non-official) can be assessed to look for events or patterns that may be related to the emerging disease.

- Conduct and review occupational health-based surveillance, particularly in health care settings, which may identify events or practices of relevance. These may include occupational hazards in infected individuals, such as among healthcare workers, laboratory workers and animal handlers or individuals subject to other environmental exposures.
- Conduct studies to test hypotheses about association with proposed risk factors, using confirmed cases and appropriate comparators drawn from the same population at the early stage of the epidemic.
- Review data from relevant 'indicator-based' surveillance systems in the affected populations relating to the period prior to the recognized start of the epidemic to look for evidence of earlier than previously recognized disease activity in this population. Disease-specific data, syndromic surveillance, any increase in medication use, and mortality data should be included.
    - Consider conducting additional analyses, including time-series analyses, if signals emerge from surveillance data suggesting the possibility of earlier disease activity.
    - Compare data from surveillance in the affected population with equivalent data from neighbouring regions and countries.
- Investigate the availability of information on patients in the geographic area(s) where initial cases were detected alongside compatible clinical illnesses in health system databases recorded in the period prior to the onset of the recognized epidemic. Use these data to look for evidence of earlier occurrence of compatible illness. If signals emerge, review the epidemiological patterns, and arrange for testing of any stored clinical samples.
    - In addition to human samples held at clinical diagnostic labs, consider those available in research laboratories and networks of surveillance and reference laboratories during the first months after cases are detected and, if necessary, conduct retrospective analyses.
- Review the availability of samples collected for other purposes, such as blood donation, and arrange for serological testing using validated methods and reagents at the time of or before first cases were detected.

Animal-human interface element

Element Scope

Studies evaluating direct and indirect exposures from wild and domestic animals should explore transmission between potential or known ancestral hosts, intermediate hosts and humans, including identification of animal species susceptible to infection with a pathogen of interest using a One Health approach. Close collaboration with FAO, WOAH, UNEP and WHO will be critical to conducting needed investigations.

Key areas of investigations

- Investigate the role of a spillover event(s) that may have led to the emergence of a novel pathogen. Any animal exposures and contact with animals should be identified and investigated.
- Identify potential disease die-offs in animals in the vicinity of the first cases that may signal the emergence or re-emergence of a pathogen with zoonotic potential. This may include domestic animals, birds, or wildlife with clinical signs, abortions, or fatalities.
- Identify potential sources of infection, such as consumption or contact with animals and animal products, hunting or farming practices, animal bites, contact with bodily fluids such as rodent urine or insect bites.
- Investigate potential reservoir hosts, such as bats, rodents, birds, and wildlife that may not display clinical signs and may be the source of the pathogen or precursor strains.
- Identify potential intermediate hosts that may act as amplification hosts or vectors.
- Define the host range through investigation of natural infections, reverse zoonoses, or infection experiments.

Recommended studies

- Studies aimed at detecting potential zoonotic pathogens in wildlife and domestic animals, including genome sequencing and isolation, should be conducted. This may include screening for specific syndromes in various species or potential reservoir species and intermediate hosts using genus PCR, virus isolation or next-generation sequencing protocols.
- Animal species identified around the initial outbreak, including animals sourced at the epicentre of the outbreak (for example, live animal markets, wet markets) and follow up investigations of trade from upstream farms, including domestic species and farmed wildlife supplying these markets, should be conducted through specific molecular or serological assays.
- Prospective serosurveys of the novel pathogen in domestic and farmed wildlife animals and humans in the geographic area where the novel potential pathogen was detected are needed to assess the risk of spillover in humans. If serologic assays are not yet available, samples should be collected and stored.
- Retrospective testing of humans and animals, including serosurveys of samples, should be performed to identify possible intermediate hosts and potential new animal reservoirs at markets, farms, slaughterhouses, etc.
- Behavioural risk studies on the human-animal-environment interface in the area where the first cases in humans were reported should include infected and non-infected groups.
- Studies of susceptibility of animal species combining laboratory receptor studies, experimental infections or reports of natural infections with a novel zoonotic virus to determine the host range and potential intermediate hosts. In addition, when possible, and once the cellular receptor of the novel emerging pathogen is known, investigate the sequences of this cellular receptor in a wide array of animal species, including domestic and farmed wildlife species.
- Conduct interviews and investigations of animal husbandry sites and breeding sites of any suspected reservoir or intermediate hosts (domestic species or hunted/farmed wildlife species). Animals should be tested, and people employed in these breeding facilities or downstream value chains should be identified and serologically tested. Studies should include validation of laboratory methods, sampling method (including number of samples) and positive controls.

- Review all available data generated by the above-recommended studies to design advanced analytics, mathematical/statistical models to understand the spillover dynamics of a novel zoonotic pathogen from wildlife to domestic animals and humans.
- Create or adapt databases to gather epidemiological, genomic and molecular epidemiology data from animal pathogens (new and known) and human pathogens and develop algorithms to better monitor their relatedness and ancestor in real-time.
- Conduct a multi-sectoral risk assessment of the possible role of the food chain of animal origin, including the cold-chain process, to assess the possible introduction of the novel pathogen into the human population through consumption of animal products.
- Investigate the potential for reverse zoonoses in animals with close contact with humans infected with the novel pathogen, including domestic animals, farmed animals, and wildlife kept in captivity, such as in zoos or other animals with regular human contact.

Environmental and ecological studies element

Element scope

Determining the persistence of a pathogen in different environments can help understand the potential for transmission pathways of a pathogen. These pathways may be dynamic from an environmental perspective, occurring through surface contamination, transmission through inhalation of infectious particles through the air, water-borne and food-borne pathways, and arthropod vectors.

Studies focussed on human or animal sampling should systematically include environmental samples at the same location, varying according to the type of pathogen.

Key areas of investigation

- Data collection: timely environmental sampling (including retrospective) should include places with a high risk of exposure/transmission, such as live animal (wet) markets, animal husbandry and breeding sites, farms, slaughterhouses; dairy, fur, and other animal-derived product manufacturing sites; and vehicles and equipment used to transport animals and wastewater.
- Arthropod host/vector surveillance should be conducted around areas where cases were detected to list vectors present, host feeding patterns and vector competency studies.
- Transmission routes: existing evidence, including reports and publications of past similar outbreaks, should be examined, as should environmental information (e.g. precipitation, temperature, and relative humidity), human and animal population distribution, and demographics.

Recommended studies

- Identify essential species for targeted intervention and potential reservoirs/intermediate hosts involved in transmission and/or spillover.
- Locate, collect and analyse any relevant environmental samples (water, air, vector, surface and waste) in the geographical area in proximity to where transmission initially occurred/pandemic may have started.

- Consider the impact of environmental or climatic changes that may affect vector intensity and geographic range.
- Determine the capacity of the pathogen to stay viable in the air or on various surfaces and under various chemical and physical conditions (such as pH, ultraviolet light, temperature, or on various surfaces).
- Perform retrospective studies on wastewater or prospective studies on environmental samples collected from wastewaters for other programmes or purposes such as SARS-CoV-2 or poliovirus monitoring, if applicable.
- Determine vector abundance, distribution and densities at outbreak sites to establish identities and diversity of potential disease vectors and their population threshold for disease transmission, if applicable.
- Evaluate vector potential and host preference of vectors to identify species for targeted intervention and potential reservoirs/intermediate hosts involved in transmission and/or spillover.
- Screen vectors for the identified pathogen by genus or specific PCRs to identify the infection rate. Characterize pathogen-vector interactions at outbreak sites and analyse vectors possibly involved in disease transmission.

Genomics and phylogenetics element

Element scope

Genomic and phylogenetic studies facilitate the identification of the origins of novel pathogens by providing viral/bacterial genomics information and both spatial and temporal dynamics of pathogen circulation. This information should be cross-cutting and trace closely related viruses and other pathogens among animal hosts, human samples and the environment. These investigations aim to define the potential evolutionary relationships in the emergence of novel viruses or variants.

Key areas of investigation

- Sequencing of infected samples during the early stages of an outbreak allowing for genomic studies and comparison of sequences with known organisms or variants/subtypes of pathogens.
- Identification and population dynamics of the pathogen and rates of pathogen spread, allowing for tracing back and establishing chain of transmission where possible.
- Searching of databases such as GISAID, Genbank, and other national databases that host real-time genome sequences, metadata, and epidemiological and clinical data for data sharing and cross-referencing purposes.

Recommended studies

- Collect human clinical samples, animal samples from potential reservoir hosts, intermediate hosts and animals with clinical signs; and environmental samples at the time of emergence for nucleic acid extraction and submit genomic sequencing to platforms in a timely manner.
- Conduct genomic analysis surveillance using sequencing and metagenomics throughout the

outbreak/epidemic/pandemic to define the evolution and identify variants of concern in humans and animals.

- Conduct metagenomics investigations using high-throughput sequencing on available samples to identify circulating variants and cryptic mutations that may signal novel variants that are potentially of animal origin.
- Review all available genomic data and metadata linked to any retrospective studies conducted focusing on epidemiology in humans, animals and environment samples.
- Analyse data and make it easily analysable and visualizable through the use of online tools to predict the potential spread of virus variants and identify new viruses.
- Include phylogenetic analysis and evolutionary studies to determine the rate of evolution and calculate the time of emergence.
- Identify closely related and ancestral strains in animals that may predict the origins of the pathogen and transmission trajectory of new emerging or re-emerging pathogens and novel variants.

Laboratory biosafety and biosecurity element

(developed in collaboration with the WHO Technical Advisory Group on Biosafety)

Element scope

A possible breach of laboratory biosafety or biosecurity may be caused by an accidental event or a procedural or engineering failure that results in the infection of staff working in a laboratory, when they are handling animals or collecting and handling specimens, either in a field or laboratory setting. Such breaches of laboratory biosafety or biosecurity may also result in the release of pathogens from a laboratory into the environment or the community via direct or indirect means. Note that the deliberate or intentional release of pathogens from laboratories is not covered in this framework.

During the initial stage of an outbreak of an unusual cluster, it can be challenging to determine if the outbreak is due to a deviation of laboratory biosafety or biosecurity measures. This is because the laboratory or nearby facility may not be the first place where the outbreak is detected. Therefore, it is essential to investigate and determine as early as possible if a breach in biosafety and biosecurity occurred in a research facility to have access to the workers and any necessary samples. The initiation of biosafety and biosecurity investigations is closely linked to the results of early investigations and the local or regional epidemiological situation.

Key areas of investigation

- National regulatory requirements applicable to the laboratory and pertinent to laboratory biosafety and laboratory biosecurity including oversight mechanisms
- Presence of laboratories or other facilities working in the geographic area where novel pathogens of unknown origin or unusual pathogens have emerged or where or any other epidemic-prone pathogens have re-emerged (including extinct or exotic pathogens)
- Presence of research entities that carried out field studies to collect animal or environmental samples that could potentially contain the emerging pathogen.

Recommended studies

- Analyse all available laboratory information and documentation on the biological risk management system in place for the facilities according to the topics (such as training, certification and maintenance) covered by national/regional legislation or the WHO Laboratory biosafety manual *(3)*.
- Identify and investigate the type and scope of research activities performed with novel pathogens of unknown origin, pathogens that have re-emerged from extinct or exotic sources, or with any other similar epidemic-prone pathogen in the local area where first cases appeared.
- Conduct a laboratory investigation in all relevant laboratories in the vicinity (range will need to be discussed with relevant authorities) to better understand the biological risk management system in place, considering the following descriptions and questions (note that the list is not exhaustive):
    - Organizational chart describing Individual's responsibilities with responsibility for laboratory biosafety and biosecurity
    - Roles and responsibilities of staff responsible for biosafety and laboratory biosecurity (e.g. biosafety officer, line management, reporting system)
    - Numbers of staff involved in biosafety management and those performing laboratory or animal work
    - Provision of a biological risk management assessment template at the facility
    - Descriptions and dates of:
        - institutional laboratory biosafety committee including membership, roles and responsibilities, example of a case
        - training and competency assessment for laboratory staff, including approvals for independent working
        - occupational health and safety programme to monitor staff health and review any sick staff within a defined period before the earliest cases were detected
        - accident / incident investigation and time series and reporting systems, corrective actions; handling non-conformities
        - facility operation and maintenance organization (including monitoring and handling engineering failures, and frequency of maintenance)
        - methodology and frequency of certification for primary biological safety cabinets and secondary containment (high-efficiency particulate air filters placed before exhaust fans)
        - scenarios, plans and exercises to prepare for an emergency response
        - inventory management systems (access, control, review mechanism)
        - laboratory activities normally performed (i.e., in vitro, virus isolation, animal experiments).

Reporting of findings

If a novel pathogen is detected and has spread into the human population, local investigators should notify national authorities as soon as possible in accordance with national policies and to WHO via the International Health Regulations (2005). As more information is gathered through the undertaking of studies and scientific investigations, additional reports should be made available immediately to relevant partners and internationally *(2)*.

Reports/publications should include an evaluation of the assessments undertaken to achieve a holistic understanding of the investigated event, including the nature of the pathogen, the potential mechanisms leading to the first human cases, and related implications. Investigations and studies often involve qualitative and quantitative methodological approaches and should involve input and findings from a wide range of disciplines and specialties, including clinical medicine, infectious diseases, epidemiology and public health, microbiology, infection prevention and control, laboratory biosafety, molecular biology, veterinary, wildlife and entomological science, environmental science, social science and anthropology, bioinformatics, statistics and data science.

Scientific publication (pre-prints and peer-reviewed) is encouraged but should not delay or present obstacles to sharing of all discoveries with national and international authorities.

Typically, report sections should include:

- Executive summary
- Background and context for the initial outbreak
- Detailed methods for how the investigations were carried out and any laboratory testing methods
- Early events/timelines leading to the recognition of the outbreak
- Initial control measures and their impact, if known
- Extent of case finding, including development of diagnostics and assessment of the initial descriptive epidemiology, including methods for case finding
- Results/findings of all investigations and targeted studies, including early investigations, human studies, animal, environmental and ecological studies, genomic studies, and biosafety and biosecurity investigations
- Assessment of quality, comprehensiveness, and limitations of the investigations
- Key conclusions and any uncertainties
- Suggestions/recommendations and timelines for further studies/investigations.

Draft reports should be shared with relevant stakeholders for discussion and action, primarily to stop onward transmission or new spillover introduction events. The investigative groups will need to consider input from all reports and incorporate them where appropriate while ensuring any changes are fully consistent with existing evidence.

Following the publication of the first reports, new information and discoveries will continually come to light. This will necessitate constant updating and new versions of reports that may alter their conclusions. The authors of the reports should anticipate and welcome this ongoing iterative process. Updated reports must be developed and released as any further investigations are carried out and completed.

Next steps for implementation and feedback

Given the pandemic risks we collectively face, it is critical that all Member States remain vigilant about the possible emergence or re-emergence of a pathogen with epidemic or pandemic potential. It is intended that in the face of such an event, this framework will provide necessary guidance for conducting comprehensive scientific investigations on the origins of such pathogens.

Timely origins investigations with immediate attention to the site or sites where an infectious disease caused by a novel pathogen was first detected could potentially avert a massive public health crisis. As has been stressed throughout this Framework, success will hinge on two fundamentals: 1) close collaboration between appropriate government, public health agencies, research institutions and local partners in the country where the pathogen is first identified and openness to the support of international organizations such as WHO and its technical advisory groups; and 2) full transparency regarding all findings so that immediate mitigation measures can be taken to prevent a pandemic.

WHO encourages feedback on the use of this framework, which can be used for the purpose of revisions and updates.

Glossary

Animal-human interface: A continuum of contacts and interactions among people, animals, their products, and their environment(s); in some cases, facilitating transmission of zoonotic pathogens or shared health threats.

Biosecurity: Principles, technologies and practices that are implemented for the protection, control and accountability of biological materials and/or the equipment, skills and data related to their handling. Biosecurity aims to prevent their unauthorized access, loss, theft, misuse, diversion or release.

Biosafety: Containment principles, technologies and practices that are implemented to prevent unintentional exposure to biological agents or their inadvertent release.

Cluster: An aggregation of relatively uncommon events or diseases in space and/or time in amounts that are believed or perceived to be greater than that expected by chance.

Disease X: Disease X refers to indicate a new unknown pathogen that could cause a serious epidemic.

Emerging infectious disease: A disease that has either has appeared and affected a population for the first time, or has existed previously, but is rapidly spreading, either in terms of the number of individuals getting infected, or to new geographical areas.

Epidemic: The occurrence in a community or region of cases of an illness, specific health-related behaviours, or other health-related events clearly in excess of normal expectancy. The community or region and the period in which the cases occur are specified precisely. The number of cases indicating the presence of an epidemic varies according to the agent, size and type of population exposed, previous experience or lack of exposure to the disease, and time and place of occurrence.

Genomics:The study of the genome, closely related to genetics and molecular biology. It began with studies to map the positions and the sequence of individual genes in chromosomes and with attempts to identify the functions of as many genes as feasible, including in particular those that are or seem to be essential for normal healthy development and metabolic functions and those that appear to be associated with occurrence of abnormalities of many kinds.

Index case/case zero: The first case identified by health authorities within an outbreak or a cluster.

Intermediate host: The animal, insect, or occasionally human in which an infectious pathogen passes a developmental stage preceding the adult stage. Insects such as mosquitoes, ticks, and blackflies; freshwater snails; animals; and fish are usually the intermediate hosts of pathogens that cause human disease.

Novel pathogen: an emerging pathogen not previously detected in the human population.

One Health: an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. It recognizes that the health of humans, domestic and wild animals, plants, and the ecosystem are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate change, preventing disease outbreaks, and contributing to sustainable development.

Origin: the ancestral host or environment from where a pathogen has evolved.

Pathogen spillover: the event in which a pathogen is transmitted from one species to another species, usually from animals to humans (zoonoses) but also from humans to animals (reverse zoonoses), which is called "spillback".

Quadripartite: A One Health memorandum of understanding between the four international agencies, the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (WOAH, formerly OIE), the United Nations Environment Programme (UNEP) and the World Health Organization (WHO) was constituted to strengthen cooperation to sustainably balance and optimize the health of humans, animals, plants and the environment.

Phylogenetics: Describing a system of classification of organisms that aims to show their evolutionary history.

Reservoir: Any animal, person, plant, soil, substance – or combination of any of these – in which a zoonotic disease agent normally lives and multiplies, and for which it primarily depends on for its survival. It is from the reservoir that the infectious substance is transmitted to a human, animal, or other susceptible host.

Risk assessment: a systematic process for gathering, assessing and documenting information to assign a level of risk.

Source: any animal species or population susceptible to the infection, be it a reservoir or not.

Zoonotic disease/Zoonoses: Infectious diseases that can be spread between animals and humans either directly from animals or through arthropod vectors.

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Annex 1 - About the Scientific Advisory Group for the Origins of Novel Pathogens (SAGO)

The SAGO, formed in November 2021, is a scientific advisory group to WHO, with observation by WOAH, FAO, and UNEP. It serves to advise WHO on technical and scientific considerations regarding emerging and re-emerging pathogens and is composed of experts acting in a personal capacity (see the terms of reference of the SAGO). It was established in accordance with the WHO Regulations for Study and Scientific Groups, Collaborating Institutions and Other Mechanisms of Collaboration.

As a core component of its mandate, SAGO was tasked with developing a global framework to define and provide guidance on the steps to be taken by regional, national, and/or local health authorities to understand how a pathogen has infected a human population. Understanding how a pathogen is transmitted can prevent further transmission in human or animal populations that could otherwise result in large-scale epidemics or pandemics. This includes forming multidisciplinary and international scientific teams to investigate the emergence or re-emergence of a novel pathogen in the human population and studies that need to be performed to better understand the mechanisms surrounding the pathogen's emergence into a naïve population and its ability to proliferate and cause disease.

Annex 2 – Information about susceptible species and/or hosts

Below is a table of pathogens with epidemic/pandemic potential. When considering implementation of the framework, a full list of pathogens is available in the Pathogens Priorization* document.

Taxa	Examples of pathogens of concern	Understanding of confirmed or probable host and/or susceptible species	Syndrome/category
Viral Haemorrhagic Fevers with animal hosts			
Filoviridae	- Ebola virus - Marburg virus	Fruit bats; duikers; non-human primates; swine Bats; humans	Ebola virus Disease Marburg virus Disease
Order: ***Bunyavirales:*** *Arenaviridae*	**Africa (Old World)** - Lassa virus - Lujo virus **South America (New World)** - Junín virus - Guanarito virus - Machupo virus - Brazilian haemorrhagic fever virus	Rodents; humans	VHF Lassa (Haemorrhagic) Fever Lujo Haemorrhagic Fever Argentine haemorrhagic fever Venezuelan haemorrhagic fever Bolivian haemorrhagic fever Brazilian haemorrhagic fever
Hantaviridae	- Old World hantaviruses - New World hantaviruses	Rodents Rodents; (Humans: Andes virus only)	Haemorrhagic fever with renal syndrome (HFRS) Hantavirus pulmonary syndrome (HPS)
Arboviruses			
Bunyavirales: *Phenuiviridae*	- Rift Valley Fever virus	Livestock (cattle, sheep, goats, and camels); wildlife (buffalo, antelope)	Meningo-encephalitis, VHF
Nairoviridae: *Peribunyaviridae* Genus: *Orthobunya virus*	- Crimean-Congo haemorrhagic fever (CCHF) virus - Oropouche virus (OROV) - Shunivirus, - Batai virus, - Ngari virus, - Jamestown Canyon virus, - Tahyna virus - Keystone virus, - California encephalitis virus	Wildlife: rat, hare, hedgehog, ostrich Livestock: sheep, goat, cattle Sloth, marsupials, bird, non-human primate Horse Bird, livestock (ruminant) Small ruminants Deer Rodent, lagomorph, hedgehog Deer, raccoon Deer, horse	ILI, VHF, liver failure ILI, vomiting, arthralgia, gastrointestinal symptoms Meningitis ILI, gastro-intestinal symptoms VHF Rarely meningo-encephalitis ILI, meningo-encephalitis, rash, fever, pneumonia Rash, fever ILI, encephalitis

*https://www.who.int/publications/m/item/pathogens-prioritization-a-scientific-framework-for-epidemic-and-pandemic-research-preparedness

Taxa	Examples of pathogens of concern	Understanding of confirmed or probable host and/or susceptible species	Syndrome/category
Flaviviridae Genus: Flavivirus	**Mosquito borne** - Yellow Fever virus - Dengue (1-4) virus - Zika virus - Wesselsbron virus - Spondweni - Sepik - Japanese encephalitis - West Nile virus - St Louis encephalitis virus - Murray Valley encephalitis virus - Usutu virus **Tickborne encephalitis** - Omsk haemorrhagic fever virus - Alkhurma virus, Kyasanur - Forest virus - Powassan virus - Louping Ill virus	 Non-human primates, humans Non-human primates, humans Monkey Wildlife, rodent, monkey,livestock Non-human primates Pigs, birds Birds Birds Birds Birds Rodent Camel, sheep Rodent, non-human primate Rodent, deer Sheep	 VHF, Jaundice Dengue fever, Severe Dengue, VHF ILI, Guillain-Barré, microcephaly Myalgia, arthralgia Myalgia, arthralgia Fever, encephalitis Fever, encephalitis
Togaviridae Genus: *Alphavirus*	- Chikungunya virus - O'Nyong–Nyong virus, - Middelburg virus - Sindbis viruses - Ross River virus - Venezuelan equine encephalitis (VEE) virus - Eastern equine encephalitis (EEE) virus - Western equine encephalitis (WEE) virus - Mayaro virus - Una virus - Getah virus	Bird, non-human primates; humans Horse Birds Rodent (enzootic), horse (epizootic) Bird (mammal, reptile) Birds, mammals, horse Non-human primates Birds, horses Birds, pigs, horses	Arthralgia Arthralgia, rash Neurological, meningo-encephalitis Arthralgia, rash Arthralgia, rash ILI, encephalitis ILI, encephalitis ILI, occasional meningo-encephalitis ILI, arthralgia Mild No illness
Other viral taxa of potential concern			
Poxviridae	- Variola virus - Mpox virus	Humans Rodents, Squirrels, non-human primates	Skin lesions, haemorrhagic Skin lesions, pneumonia, encephalitis
Paramyxoviridae *Henipaviruses*	- Nipah virus - Hendra virus	Bats, Pigs Bats, Horses	Pneumonia, encephalitis

Taxa	Examples of pathogens of concern	Understanding of confirmed or probable host and/or susceptible species	Syndrome/category
Orthomyxoviridae *Alphainfluenzavirus*	- Influenza A viruses (Avian; Swine)	Water birds Poultry Swine, cattle, Horses, dogs, Humans (rapidly expanding range of species affected)	Influenza-like illness, pneumonia, respiratory failure
Coronaviridae	- SARS-CoV-1 - SARS-CoV-2 - MERS-CoV	Bats, civets, raccoon dog Confirmed in multiple mammalian species, including carnivores, mink, deer, non-human primates, hamsters Camels	Severe acute respiratory syndrome COVID-19; anosmia, pneumonia, sepsis, ARDS, kidney/respiratory failure, Long COVID Pneumonia, ARDS
Lentivirinae	- HIV; SIV; HTLV	Humans, non-human primates	Acquired immunodeficiency syndrome
Picornaviridae Enteroviruses	- Poliovirus (type 1-3) - Coxsackie virus (A/B) - Enterovirus 68	Humans	Paralytic poliomyelitis aseptic meningitis febrile disease, encephalitis, myopericarditis, pleurodynia, pneumonia, hepatitis Respirarory, meningo-encephalitis, acute flaccid myelitis

Bacterial pathogens of concern: Emerging and re-emerging zoonotic bacteria or drug-resistant bacteria, which correspond to the National Institute of Allergy and Infectious Diseases list of A, B and C pathogens. Relevance to SAGO will be determined on a case-by-case basis.

Examples of pathogens of concern	Disease	Understanding of confirmed or probable host and/or susceptible species	Syndrome/ category
Bacillus anthracis (A)	Anthrax	Livestock; wildlife	Skin sores, flu like symptoms, vomiting; meningitis, shock
Clostridium botulinum (A)	Botulism	Humans	Loss of nerve functions, respiratory and muscular paralysis
Yersinia pestis (A)	Plague	Rodents, humans	Constitutional symptoms, bleeding, inflamed lymph nodes, Systemic infection
Francisella Tularensis (A)	Tularaemia	Wildlife (mainly rabbit), domestic cats, humans	Skin ulcers, enlarged lymph nodes, fever, local lesions, respiratory symptoms
Burkholderia pseudomallei (B)	Melioidosis	Wildlife and farm animal	ILI, hepatitis
Coxiella burnetii (B)	Q fever	Farm and domestic animal	Systemic infection
Brucella species (B)	Brucellosis	Farm and domestic animals, humans	Flu-like symptoms, fever
Burkholderia mallei (B)	Glanders	Horses, mules, donkeys, dogs, and other domestic animals, humans	Constitutional symptoms, nasal discharge, light sensitivity
Chlamydia psittaci (B)	Psittacosis	Wild and domestic birds	Constitutional symptoms, dry cough
Clostridium perfringens (B)	*Clostridium perfringens* (Epsilon toxin)	Foodborne: poultry, cattle, pigs	Diarrhoea, abdominal cramps and bloating
Staphylococcus aureus (B)	*Staphylococcus Enterotoxin* B (SEB)	Livestock	Nausea, diarrhoea, vomiting, respiratory symptoms, toxic shock syndrome
Rickettsia prowazekii (B)	Epidemic typhus	Flying squirrels	Fever, flu-like symptoms, vomiting, nausea, rash, complications affecting multiple organs
Ehrlichia (multiple species) (-)	Ehrlichiosis	Dogs, deer, Human	Fever, constitutional symptoms, nausea, confusion, rash

Examples of pathogens of concern	Disease	Understanding of confirmed or probable host and/or susceptible species	Syndrome/ category
E.coli (B)	Diarrhoeagenic *E. coli*	Human, animals	Diarrhoea, fever
Vibrio cholerae (B)	Cholera	Human	Severe diarrhoea and vomiting, shock
Shigella species (B)	Shigellosis	Human	Fever, diarrhoea, haematochesia, abdominal cramps, Tenesmus
Salmonella species (B)	Salmonellosis	Poultry, human	Fever, abdominal pain, diarrhoea, vomiting, fatigue, and intestinal perforation in severe cases
Listeria monocytogenes (B)	Listeriosis	Mainly foodborne	Febrile illness, respiratory and gastrointestinal symptoms
Campylobacter jejuni (B)	Campylobacteriosis	Poultry, cattle, human	Fever, abdominal cramps, bloody diarrhoea, (rarely Guillain-Barré)
Yersinia enterocolitica (B)	Yersiniosis	Humans	Diarrhoea, haematochezia
Mycobacterium tuberculosis (C)	Tuberculosis	Humans	Respiratory symptom, tuberculosis
Protozoa (B)	*Cryptosporidium parvum* and *Giardia lamblia*	Human, wildlife	Diarrhoea
	Cyclospora cayatanensis	Shellfish, dog, non-human primate, mainly human	Diarrhoea
	Entamoeba histolytica	Felidae, rodent, human	Diarrhoea
	Toxoplasma gondii	Foodborne or in domestic animals (cats), humans	Abdominal pain, ILI, encephalitis, transplacental: brain and eye abnormalities
	Balamuthia mandrillaris	Humans	Skin ulcers, febrile illness, encephalitis
	Naegleria fowleri	Humans	Fever, nausea, vomiting, meningitis
Fungi (B)	Microsporidia (Microsporidiosis)	Domestic, wild animal	Varies according to species and route of transmission

Examples of pathogens of concern	Disease	Understanding of confirmed or probable host and/or susceptible species	Syndrome/ category
Prions (C)	Transmissible Spongiform Encephalopathies (TSE) - Bovine Spongiform Encephalitis (BSE) - Other TSEs	Cattle Wildlife (deer, antelope) and farmed animals (sheep, camel)	Variant Creutzfeldt-Jakob disease (vCJD)

Annex 3 – List of sample materials recommended for sample collection during infectious disease outbreaks and/or origins investigations

Type of sample	Zoonotic virus	Vector-borne virus	Other? (Food-borne/Air-borne/STDs)
Human cases	Biological material from early human cases (and controls), e.g. throat swabs, blood and serum (and CSF if indicated); post-mortem samples (visceral organs, liver, spleen, kidney); brain; lung.	Blood or serum samples from the cases for identification of causative agent (RDTs and PCR); CSF; urine; post-mortem tissue (liver; spleen; brain; spinal cord). Also, body fluids in certain diseases like Crimean-Congo haemorrhagic fever or other haemorrhagic fevers.	**Food-borne:** blood samples; stool samples; hand swabs; microscopy; culture and sensitivity (C/S). **Airborne:** throat swabs; nasal swabs; oropharyngeal swab; blood samples; body fluids in some diseases; STDs; blood samples for C/S; urine samples for C/S; semen samples for C/S and body fluids in certain diseases; genetic sequencing in some diseases. **STDs:** blood samples for C/S; urine samples for C/S; semen samples for C/S and body fluids in certain diseases; genetic sequencing in some diseases.

Type of sample	Zoonotic virus	Vector-borne virus	Other? (Food-borne/Air-borne/STDs)
Animal/source	Biological material from domestic/wild animals/ animal products at/ nearby epicenter and relevant geographical locations guided by early investigations: structured sampling of serum, throat/anal/cloacal swaps, post-mortem tissues visceral organs/ brain/lung from sick animals; animal residues from slaughtering.	Vector surveillance through vector control programme (entomologists/vets etc.; vector insectary and reference lab for genetic sequencing of the vector for species identification and geographical relevance; blood meal analysis on engorged females may identify host feeding habits of vectors; animal reservoirs /amplification hosts through syndromic surveillance for febrile/ neurological/abortions cases; or Cloacal or oral swabs/EDTA blood for PCR/serum for serology.	**Food-borne:** food samples relevant to the outbreak for analysis; water samples for analysis. **STDs:** contact tracing for sample collection.
Environmental	Sampling of environments surfaces, drainages, animal cages, waste water of suspected epicentre (e.g. market, hospital, mine etc.).	Water sampling for larvae identification and density; environmental survey for vector density and identification.	**Food-borne:** environmental samples including surfaces, utensils, cutlery and cutting board surfaces; raw food (ingredients sampling). **Airborne:** samples of the surfaces and other items related to the case; air samples for pathogen density measurements; drainage water samples for environmental circulation assessment of the organism.

Annex 4 - Assessment tools to assess breaches in biosafety and biosecurity for laboratory or field activities or other activities where biological risks are present

Many countries have biosafety regulations and oversight mechanisms in place, including inspection checklists. These should be consulted as they form the basis of a biosafety and biosecurity investigation.

Other tools and checklist to be covered by an investigation includes the WHO Laboratory Biosafety Manual, 4th edition and its associated monographs *(3)*.

The approaches described in Variola virus repository safety inspections reports provide the most solid basis for structuring an investigation in case of an outbreak of an extinct or exotic pathogen (see the WHO document, Variola virus repository safety inspections).

The Biorisk management standard (ISO 35001:2019) outlines all relevant biosafety and biosecurity issues and can also be used as a reference for an investigation *(4)*.

Every framework used for an investigation must be applied in an objective, scientifically rigorous, comprehensive, credible and reasonable manner. The current state of the art and science should be considered in the assessment:

- Develop a biosafety and biosecurity assessment tool
- This tool can be used in future for in investigating the origin of novel pathogens. The protocol should be developed with support of the WHO Technical Advisory Group on Biosafety (TAG-B) *(3)*.
- To investigate whether biosafety and biosecurity are well managed within a laboratory and during field studies or associated activities, detailed information should be obtained and analysed in a structured and systematic way.
- The approaches detailed in Variola virus repository safety inspections provides the most solid basis for structuring such evaluations.

Wherever this framework is used, it must be applied in an objective, scientifically rigorous, comprehensive, credible and reasonable manner. The current state of the art and science should be considered in the assessment.

References

1. World Health, O., *Protocol to investigate non-seasonal influenza and other emerging acute respiratory diseases*. 2018, World Health Organization: Geneva.

2. World Health, O., *International health regulations (2005)*. 2008: World Health Organization.

3. World Health, O., *Laboratory biosafety manual*. 4th ed ed. Laboratory biosafety manual, fourth edition and associated monographs;. 2020, Geneva: World Health Organization.

4. ISO. *Biorisk management for laboratories and other related organisations*. 2019 [cited 2023 September]; Available from: https://www.iso.org/obp/ui/#iso:std:iso:35001:ed-1:v1:en.