Co-funded by
the Health Programme
of the European Union

Clinical Management Guidelines for Biological and Chemical Agents

Deliverable 5.2

Due Date of deliverable: 31st July 2024

Actual submission date:19th October 2024

Disclaimer:

The document has been developed under task 5.2 "Updating clinical guidelines/memory aid sheets or expert statements for the recognition, case management and containment of patients exposed to highly pathogenic biological and chemical agents.

This report arises from the TERROR Joint Action, which has received funding from the European Union through the European Health and Digital Executive Agency of the European Commission, in the framework in the framework of the Third Health Programme 2014-2020. The European Commission is not responsible for the content of this report. The sole responsibility for the report lies with the authors, and the European Health and Digital Executive Agency is not responsible for any use that may be made of the information contained herein. The authors are not responsible for any further and future use of the report by third parties and third-party translations.

Authors:	Tom Gaulton, Martina Spaziante, Bernadette Palyi, Daniele Lapa, Martina Rueca, Serena Vita, Arne Broch Brantsæter, Siri Feruglio, Marte Petrikke Grenersen, Judit Henczkó, Jartrud Wigen Skjerdal, Mari Asphjell Bjørnaas, Francesco Vairo
Date:	23/ 09/2024
Version History:	Version number1, 15/11/2023, draft Version number2, 13/06/2024, draft Version number3, 23/07/2024, draft Version number4, 23/09/2024, draft Version number5, 18/10/2024, final

Project details

Project short name:	JA TERROR
Project full name:	JA TERROR – Strengthened preparedness and response to biological and chemical terror attacks
Grant Agreement no :	101003855
Project duration:	48 months
Project timeframe:	1/1/20 – 12/31/24
Coordinators:	Bengt Skotheim, Bjørn Jarle Wiger
Title:	Intentional Use of Biological and Chemical Agents: Clinical Management Guidelines
Work Package:	WP5
Date of Document	18/10/2024

Reviewing partner:	All

Document details

Status of the Document:	Final version
Dissemination level:	Public

Contents

Abbreviations

ARDS - acute respiratory distress syndrome
BAL -bronchoalveolar lavage
CPXV - cowpox virus
CT - Contact Tracing
DFA - direct fluorescent antibody assay
ECDC - European Centre for Disease Prevention and Control
EDTA - Ethylenediaminetetraacetic acid
EM - Electron Microscopy
EMA - European Medicine Agency
EU – European Union
EWRS - European Warning and Response System for communicable diseases and other threats to health
HEPA - High Efficiency Particulate Air
IHC - immunohistochemical assay
IVIG - Intravenous Vaccinia Immunoglobulin
JRC - Joint Research Centre
Mpox - monkeypox
MPXV - monkeypox virus
NAAT - Nucleic Acid Amplification Testing
OPXV - orthopoxvirus
PCR - Polymerase Chain Reaction
PPE - personal protective equipment
SARI - Severe acute Respiratory infection
UN – United Nations
USA - United States of America
VACV - vaccinia virus
VARV - variola (smallpox) virus
VTM - Viral Transport Media
WHO - World Health Organization
ARDS - acute respiratory distress syndrome
BAL -bronchoalveolar lavage
CPXV - cowpox virus

Executive summary

Deliverable 5.2 of Joint Action TERROR: Health preparedness and Response Planning to Biological and Chemical Terrorist attacks.

The document provides detailed guidelines for the clinical management of individuals exposed to two highly pathogenic biological agents and two highly toxic agents that could be used in a terror attack. The biological agents, orthopoxviruses and *Yersinia pestis,* and the chemical agents, chlorine and nerve agent, were selected based on consensus between WP partners. The guidelines were developed through literature review and expert consultation (remote consultation and workshops). This document also provides information on risks associated with the agents, regarding the management of an incident, including discerning between a deliberate and natural/accidental incident and elements of the response to terror attacks (including incident management, communication, and cross-sectoral cooperation). The clinical management guidelines for each agent include clinical presentation/signs and symptoms, clinical management of exposed people, and control measures. Additional information on the agents detailed in the guidelines are included in Annexes to this report.

Variola virus, which belongs to the genus *Orthopoxvirus*, is the aetiological agent of smallpox and is listed among the most significant bioterrorist threat agents and is classified by the CDC as a Category A select agent. It is estimated that smallpox caused more than 500 million human deaths during the 20th century. The disease was declared successfully eradicated by the World Health Organization's (WHO) in 1980. However, variola virus remains stored in two WHO Collaborating Centres under maximum containment. The accidental or deliberate reintroduction of variola virus into the environment threatens not only public health, but also the economy and political stability of the whole world. Therefore, the known remaining variola virus stocks are subject to WHO scrutiny for the research they are subject to, and each site is regularly assessed by WHO with regards to biosafety and biosecurity.

For orthopoxviruses, vaccines constitute the most important medical countermeasure in response to a deliberate or accidental release. At least 40 nations worldwide declare to have stockpiles of smallpox vaccine. At present, 800 million doses of vaccine or more are stocked worldwide.

Y. pestis has been classified by the CDC as a high priority pathogen, a Category A select agent, that requires special precautions, particularly because of the risk of pneumonic plague, following aerosolization by bioterrorists.

Yersinia pestis is endemic in many regions around the globe. There exists no licensed vaccines for plague, but antibiotics can be used to treat and prevent infection. The document provides detailed recommendations for antibiotic treatment and prevention for both adults and paediatrics patients.

Chlorine is a commonly used industrial chemical that could also be used to cause intentional harm, e.g. it is considered a dual use chemical. Chlorine is a reactive gas that is approximately three times as heavy as air and has a characteristic odour similar to bleach. It is part of the group of chemicals called 'halogens' which include fluorine, bromine and iodine. Chlorine was used as a chemical warfare agent during World War I. The severity of the injury is dependent on the concentration in the air, duration of exposure and presence of any pre-existing cardio-pulmonary diseases. Recommendations are given on whether to decontaminate individuals along with a clear clinical pathway to determine the level of harm. Standard supportive care is recommended following exposure to chlorine.

Nerve agents are produced exclusively for use as chemical warfare agents. They are organophosphates and are acetyl cholinesterase inhibitors. A list of nerve agent and their properties are described. The symptoms of exposure are included and could be described as mild, moderate or severe, with effects presenting within seconds/minutes/hours depending on the agent, its concentration and any underlying health issues of the individual. Drug admission regimes are detailed.

Introduction

Origin, aims, and target audience of the document

This document is Deliverable D5.2 of Joint Action TERROR, produced by WP5: Health Preparedness and Response Planning to Biological and Chemical Terrorist Attacks. The document provides guidelines for the clinical management of people exposed to selected highly pathogenic biological agents and highly toxic chemical agents, which might be used in the context of a terror attack. This document also provides information, including relevant references sources, regarding incident situational awareness and aspects of management of the incident itself.

The biological and chemical agents described in this document have been chosen following the consultation with Joint Action Terror partners and in collaboration with JA external experts. Online consultations and a specific workshop with experts were organized to reach consensus.

The aims of this document are:

- To provide clinical guidelines on the management of specific agents,
- To reduce the potential health impact and adverse outcomes from incidents,
- To create a common reference document that can be adapted for other biological and chemical agents,
- To harmonise guidelines amongst EU Member States, ensuring consistent clinical management of outbreaks, decreasing the risk of variable management quality, and fostering cross-border collaboration.

The document also includes information relevant to stakeholders involved in preparedness, management/coordination and response to incidents with the intention of ensuring effective readiness and response to potential biological and chemical terror attacks. In addition to clinicians and first responders this diverse audience may include, but not be limited to:

- Public health officials and authorities
- Laboratory staff
- Representatives from other sectors outside health
- EU agencies and policy makers

Document architecture overview

The document covers elements of risk awareness and recognition of an incident caused by the release of a chemical or biological agent, and details specific considerations relevant to that response, including incident management, communication and cross-sectoral cooperation. The clinical management guidelines for each agent follow providing two biological guidelines for

orthopoxviruses and *Yersinia pestis,* and two chemical guidelines for chlorine and nerve agents which include clinical presentation/signs and symptoms of exposure, clinical management of exposed people, and control measures. The annexes contain further information on each of the agents in this guideline.

Assessing the threat: recognition and risk awareness

Intentional release

A deliberate event is defined by the WHO as an act or threat involving the intentional release of hazardous materials to cause harm. Deliberate events may be covert (unannounced) or overt (announced). Intentional release of biological or chemical agents can occur as a result of terrorism, criminal activity, or warfare.

Terrorism involves the use of violence or threats by non-state actors motivated by political, ideological, or religious goals. This differs from warfare, which refers to armed conflict between states or organized groups, typically involving military forces, with the aim of achieving strategic goals such as territorial control, regime change, or defence. Finally, crime refers to unlawful activities committed by individuals or groups, driven by personal gain, revenge, or thrill. While the primary goal of this document is to enhance preparedness and response to acts of terrorism involving the release of biological or chemical agents, it can also be useful for addressing similar situations arising from warfare, criminal activities, or accidental releases.

Bioterrorism involves the intentional release of biological agents (like bacteria, viruses, or toxins) to cause illness or death in people, animals, or plants. The goal is to instil fear, disrupt societies, or achieve political or ideological objective. Similarly, chemical terrorism involves the deliberate release of chemicals to achieve the same goals. This document addresses the human effects of deliberate release of biological or chemical agents.

Biological or chemical incidents resulting from acts of terrorism require a response from both health sector (protecting and managing the health of those exposed), security sectors (control the source and prevent the incident from worsening) and civil protection. To classify an incident at a deliberate event, two key elements must be present:

- An exposure to a hazardous substance.
- A deliberate release of that substance.

According to the UK JESIP Initial Operational Response guidelines: It is also important to be aware that any offender(s) may still be present, and responders should be aware of the possibility of

secondary attacks, but they must do everything they can to save life and reduce harm to the public. (https://www.jesip.org.uk/downloads/initial-operational-response-ior-to-incidents-suspected-to-involve-hazardous-substances-or-cbrn-materials/)

It is critical that responders are capable and equipped to recognise hazardous substance incidents, enabling them to effectively respond and save lives while ensuring their own safety.

Recognising a deliberate event

In the early stages of an incident, it may be difficult to establish what has occurred and if hazardous substances have been used, especially if the release is not announced by the perpetrator(s) (covert attack).

Release of a biological agent

The release of a biological agent typically lacks a clearly defined incident scene. This is because the incubation period, or the time from exposure to the onset of symptoms, can range from hours to days, allowing victims to move far from the initial exposure site. This may result in an outbreak without an initial cluster.

Early suspicion of an unannounced deliberate release of a biological agent requires a high level of awareness of unusual features related to the patient, the biological agent itself, or the outbreak.

- Patient: no known personal risk for the identified disease, such as
  - Contact with animal, person, laboratory, high-risk environment.
  - Travel to endemic area.
  - Profession/leisure activity (e.g. veterinarian, health care worker, farmer, hiking).
- Agent
  - Known select agent.
  - Agent not occurring naturally in the area.
  - Unusual resistance profile.
- Outbreak
  - In unusual population.
  - Unusually rapid increase in case number.
  - Occurring in atypical season.

Release of a chemical agent

A deliberate release of a chemical agent is more likely than the release of a biological agent to trigger a cluster of casualties close to the site of the release. This is because the latent period, or the time from exposure to symptoms, may be very short for chemicals. If the chemical is still present in the environment, the first responders may be at risk.

Visual indicators of a deliberate event may include all or some of the following:

- Dead or distressed people and/or animals,
- multiple individuals with unexplained symptoms such as skin, eye or airway irritation, nausea, vomiting, twitching, sweating, pinpoint pupils, runny nose, disorientation, breathing difficulties, convulsions, and death,
- the presence of hazardous or unusual materials or equipment,
- unexplained vapour or mist clouds,
- unexplained oily droplets or films on surfaces or water,
- withered plant life and vegetation,
- odd smells or tastes out of character with surroundings.

A deliberate incident may not necessarily cause casualties, although the presence of casualties is still important and should be considered alongside the above indicators when a deliberate incident is suspected.

Symptoms of exposure to a biological attack may not be present within the first minutes, hours or days of an attack occurring. Chemical releases are often, but not always, accompanied by a more rapid onset of symptoms. Casualties must therefore be removed from the area of exposure as soon as possible, then any contaminated clothing should be removed, followed by decontamination to remove any remaining hazardous substance. Removal and decontamination reduce the likelihood of ongoing exposure, but also decreases the potential for secondary contamination of emergency personnel and clinicians.

When multiple agencies are involved/present at an incident, a joint understanding of hazards and risk will enhance decision making and ensure an effective multi-agency strategy and response to safely deliver a prompt resolution. In the UK, the M/ETHANE model is an established reporting framework which provides a common structure for responders and their control rooms to share incident information.

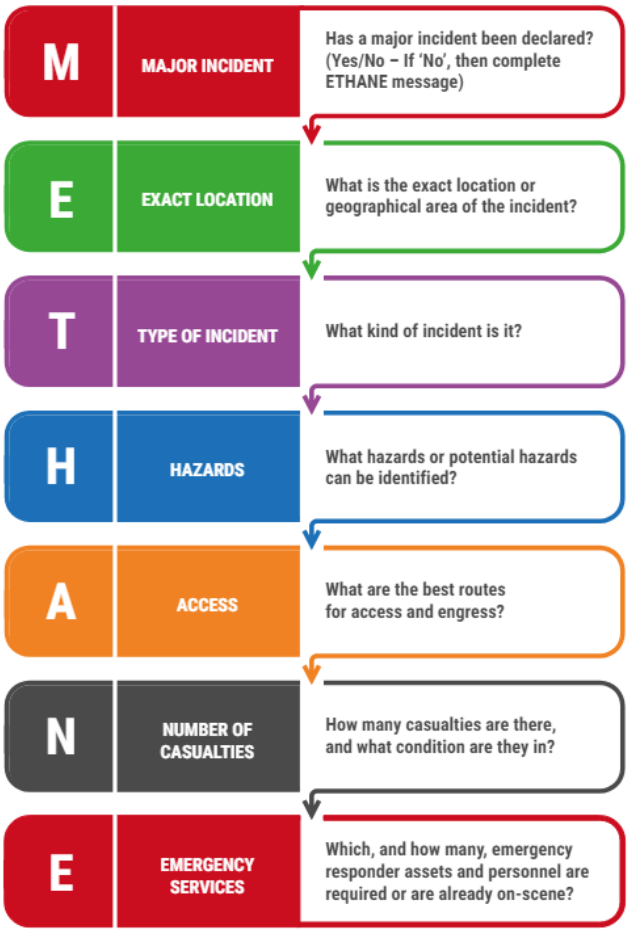

Figure 1. M/ETHANE model used to characterise incidents in the UK. Source: https://www.jesip.org.uk/downloads/joint-doctrine-guide/

Preparedness and response to intentional threats by biological or chemical agents

Incident management: public health actions

A biological or chemical terrorist attack requires a rapid response and targeted control measures. For example, in the case of biological incidents, immunisation of the general population may play a crucial role. However, vaccines may not be available, or stockpiles may be insufficient. In a chemical incident, containing the hazardous material and preventing further exposure has first priority.

In biological or chemical attacks, non-pharmaceutical countermeasures can be used to stop transmission of infection and prevent further spread of, or exposure to, chemicals. For example, social distancing and early diagnosis, can enable prompt isolation of infectious individuals in the event of release of a biological agent. Decontamination is intended to reduce patient exposure to the chemical agent and the risk of secondary contamination. For further information, see TERROR D5.3: Report on the evaluation of available non-pharmaceutical countermeasures for chemical and biological terrorist attacks.

In biological and some chemical incidents, contact tracing (CT) is important to identify those exposed to the agent and promptly identify potential secondary cases. CT can also help identify settings or population groups for which targeted interventions are needed.

For a biological incident, CT is effective at different levels:

- Individual level: Early diagnosis of infected individuals ensures they receive prompt medical attention.
- Population level: Detecting and interrupting transmission chains helps reduce the spread of the infection in society.

Public health communication aspects

A deliberate attack involving chemical or biological agents has the potential to cause substantial fear among the public. This presents a challenge for those providing information quickly after an attack, while ensuring that the message is simple to understand and likely to be followed by members of the public. Identifying in advance what people would want to know, where they would get information from, and how messages should be presented might allow communicators to ensure that their messages have the best chance of having their desired effect.

Terror attacks can make this communication difficult, as these attacks can cause significant fear amongst the population, impairing the ability of people to comprehend important information provided. This issue is also compounded by a lack of general knowledge about chemical and biological agents. Also, while some may be familiar with basic concepts which relate to the malicious use of Chemical, Biological, Radiological and Nuclear (CBRN) materials or weapons with the intention to cause significant harm or disruption, the perceived low likelihood of a terror attack occurrence may reduce the attention paid to public information. The speed at which clear and concise information needs to be disseminated and understood by the public (which may differ from traditional non-emergency public health broadcasts) also raises challenges.

Trust plays a crucial role in ensuring compliance with public messages and is important to communicate a consistent message in multiple channels.

For further information on the communication aspects involved in a terror attack, see JA TERROR D7.1: Risk and Crisis communication Guidance Tool.

Cross-sectoral cooperation

In the EU, cross-sectoral collaboration is facilitated through the EU Civil Protection Mechanism (CPM), which aims to strengthen cooperation between the EU countries and 10 participating non-EU states on civil protection to improve prevention, preparedness, and response to disasters. In the event of an emergency, any country can request assistance via the EU Civil Protection Mechanism, which can include shared stocks and joint investment and procurement strategy for medical countermeasures.

RescEU was established as a reserve of European capacities, fully funded by the EU. It includes a fleet of firefighting planes and helicopters, a medical evacuation plane, and a stockpile of medical items and field hospitals that can respond to health emergencies, including CBRN emergencies. Through RescEU, decontamination capabilities as well as detection, sampling, identification and monitoring capabilities are currently being developed.

More generally, preparedness plans with clear and defined roles and responsibilities of the different sectors can facilitate this collaboration in a terror attack. It is important that the health sector cooperates with the security and civil protection sectors, e.g. to facilitate forensics investigation; contribute to a joint surveillance system (through EWRS, or follow-up on information on the threat from the security sector which can be jointly assessed by ECDC and Interpol.

For more information on cross-sectoral cooperation, see JA TERROR D6.5 – Guiding document on cross-sectoral collaboration.

As an example from the UK, a multisectoral strategy exists for the purpose of allowing emergency responders to work together more efficiently and effectively, known as JESIP (Joint Emergency Services Interoperability Programme). The JESIP framework was designed for major emergencies (not just CBRN incidents), but can be used at all levels of response (https://www.jesip.org.uk/joint-doctrine/principles-for-joint-working/)

As during the early stages of an incident, establishing structures, protocols and resources required can take time. JESIP can facilitate an understanding of each other's roles, command and control structures and agreed models and principles, for all agencies involved in the response.

The JESIP joint doctrine lists a set of principles for joint working, they support the development of a multi-agency response and provide structure during the response to all incidents.

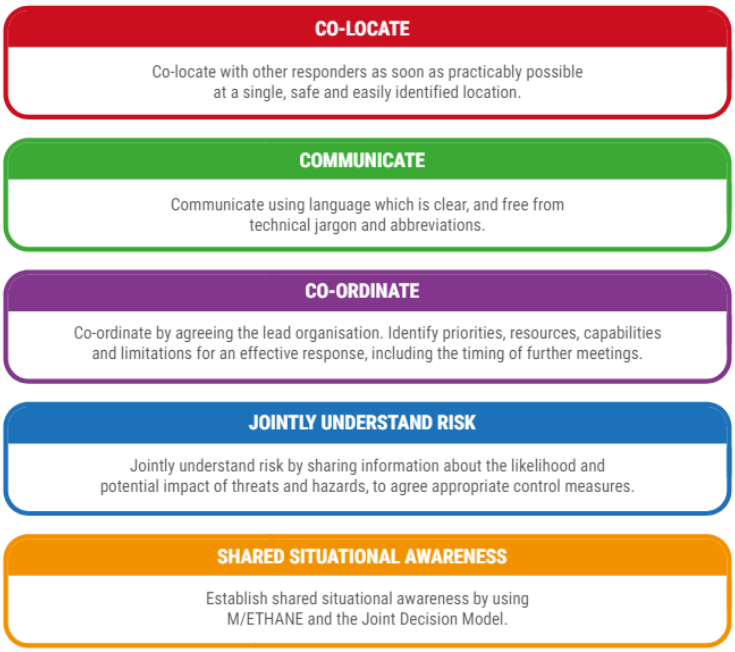

Figure 2. JESIP principles for joint working between agencies. Source: https://www.jesip.org.uk/joint-doctrine/principles-for-joint-working/

Biological agents - Orthopoxviruses

A.1 Introduction

The genus *Orthopoxvirus* comprises a number of species that can infect both animals and humans. The most well-known member of the genus is variola virus, the causative agent of smallpox. It is estimated that smallpox caused more than 500 million human deaths during the 20th century. The disease was declared successfully eradicated by the World Health Organization (WHO) in 1980.

Other notable members include vaccinia virus, which is used in the current smallpox vaccine; cowpox virus; and monkeypox virus. The most recently discovered poxvirus was isolated from a resident of Alaska. The poxviruses contain single, linear, double-stranded DNA molecules of 130-to-375-kb pairs and replicate in cell cytoplasm. They are shaped like bricks on electron micrographs and measure about 300 by 250 by 200 nm.

Orthopoxviruses and their potential for intentional release

Today, smallpox virus is stored in two locations worldwide: at the Centers for Disease Control and Prevention (CDC) in Atlanta in the USA, and at the State Research Center of Virology and Biotechnology (VECTOR) in Novosibirsk in in Russia. However, it is possible that smallpox virus may be stored elsewhere, either by neglect or intentionally. Variola virus is listed among the most significant bioterrorist threat agents and is a category A agent on the CDC list. Also, modern technology provides a way for de novo synthesis of smallpox virus. In 2018, Canadian researchers demonstrated this in a proof-of-concept study by constructing the extinct horsepox virus from chemically synthesized DNA fragments. Because smallpox has been declared eradicated by the WHO, any new case would immediately result in suspicion of a deliberate event.

Other orthopoxviruses, like vaccinia-like and cowpox-like viruses, are less likely bioterrorist agents, due to lower transmissibility and generally less severe illness compared to smallpox. Nevertheless, their potential use should not be totally dismissed, as they are widely accessible during ongoing outbreaks. Also, they may potentially mutate naturally or be intentionally modified to become more transmissible or virulent.

Certain characteristics of these viruses make them suitable for deliberate release that may have severe consequences. Most orthopoxviruses are very stable and can remain infectious in the environment for a long time. Some are also highly infectious via aerosol and easily transmissible from infected to susceptible individuals. Furthermore, their relatively long asymptomatic incubation period may make contact tracing challenging.

In the context of bioterrorism, it is conceivable that variola virus might be dispersed as a colourless, odourless and invisible aerosol using technical devices. This could be achieved through various methods, including manned and unmanned aircraft, such as drones. Additionally, smallpox virus may be spread by the contamination of commonly used materials and objects of various types, e.g. paper, fabrics, and leather, leading to indirect transmission. A rational assessment of the magnitude of casualties that a smallpox intentional attack might cause, and the identification of appropriate control measures to minimize its effects, are complicated. The time elapsed since the last smallpox outbreak limits the extrapolation of data from historical epidemics to predict impact of future outbreaks. Vaccination is an important countermeasure, but it is important to consider the potential adverse effects that some individuals may experience during a national mass vaccination campaign.

The strategy of pre-exposure vaccination of a part of the population in order to prevent the catastrophic impact of an intentional attack is animatedly debated, and the cost-effectiveness of this strategy is uncertain.

A.2 Clinical Presentations and Differential Diagnosis

Smallpox

Before its eradication, smallpox primarily spread through direct contact and respiratory droplets during extended face-to-face interactions. Occasionally, it also transmitted via the airborne route in enclosed environments, such as buildings. After a period of 7–19 days, a patient with smallpox typically develops high fever, malaise, and prostration with headache and backache, and sometimes vomiting. After about 2-4 days, a maculopapular rash appears on the mucosa of the mouth and pharynx, face, proximal extremities and trunk, and then spreads centrifugally to the entire trunk, feet and hands. Usually, it spreads to all parts of the body within 24 hours. As this rash appears, the fever begins to decline, and the person may start to feel better. By the fourth day the rash becomes vesicular and then pustular in a uniform evolution. The pustules are characteristically round, firm, and deeply embedded in the skin and have an umbilicated appearance (the lesion having a central depression). Crusts begin to form after 7–9 days: the eschars later separate, leaving pits and scars. Three weeks after the rash appears, most scabs will have fallen off. Once all scabs have fallen off, the person is no longer contagious. Bacterial superinfection of skin lesions may complicate smallpox. About 5–9% of patients with smallpox develop ocular complications, and blindness is a frequent sequela of smallpox, due to corneal, uveal, or retinal lesions. Neurological complications are uncommon, but include encephalitis, encephalomyelitis, transverse myelitis, cranial neuropathies, and Guillain-Barré syndrome. Smallpox has a case-fatality rate of 20–35% among unvaccinated individuals.

Mpox

Mpox virus (MPXV) can be divided into two genetically distinct viral clades: clade I, (formerly known as Congo Basin clade) and clade II (former West African clade). Clade I is divided into subclade Ia and Ib, and clade II into subclade IIa and IIb. The case fatality ratio of mpox has historically ranged from 0– 11 % in the general population, being higher among young children. The clade I virus is the most virulent, with human case fatality rates during outbreaks in parts of Africa estimated to be around 5% in unvaccinated persons.[1] Clade Ib has only recently been identified and less is known about its case fatality. The relevance of this virus in a bioterrorism scenario is very limited.

Clade II generally causes less severe illness and has lower inter-human transmissibility than Clade I and has a fatality rate of 1-4%. Clade IIb is responsible for the global outbreak that started in 2022 and spread primarily among men who have sex with men. The case fatality in this outbreak is around 0.1% (WHO). Meanwhile, new cases caused by clade IIa continue to be reported.

Mpox, particularly if due to clade II strains, is usually a self-limited disease with the symptoms lasting from 2–4 weeks.

The course of the infection can be divided into two periods: the prodromal period (lasting up to 5 days) is characterized by fever, intense headache, lymphadenopathy, back pain, myalgia and asthenia. Lymphadenopathy is a distinctive feature of mpox compared to other diseases that may initially appear similar (chickenpox, measles, smallpox). The second phase is characterized by the skin eruption and usually begins within 1–3 days from the appearance of fever. Lesions are firm or rubbery, well-circumscribed, deep-seated, and often develop umbilication. The evolution of lesions progresses through four stages: macular, papular, vesicular, and pustular- before scabbing over and desquamating.

Vaccinia and cowpox

Human infections with vaccinia, wild vaccinia-like viruses, cowpox, and cowpox-like viruses are most often self-limited, characterized by localized vesicular-pustular (and in cowpox, occasionally ulcerative) lesions. Fever and other constitutional symptoms may occur briefly after lesions first appear. Lesions can be painful and can persist for weeks. Immunocompromised patients or those with exfoliative skin conditions (such as eczema or atopic dermatitis) are at higher risk of severe illness or death. The relevance of these viruses in a bioterrorism scenario is very limited.

Differential diagnosis

Accurate diagnosis of smallpox is crucial, particularly in light of potential bioterrorism threats. Many other eruptive illnesses share clinical features, which could lead to misdiagnosis (Table 1).

Table 1. Smallpox differential diagnosis

Infectious disease	Characteristics
Varicella (primary infection with varicella-zoster virus)	· Most common in children <10 years · Children usually do not have a viral prodrome . Rash may involve soles and palms
Mpox	. Particularly if due to clade II strains, is usually a self-limited disease . Lymphadenopathy
Disseminated herpes zoster	· Immunocompromised or elderly persons · Rash looks like varicella, usually begins in dermatomal distribution
Impetigo (*Streptococcus pyogenes*, *Staphylococcus aureus*)	· Honey-coloured crusted plaques with bullae are classic but may begin as vesicles · Regional, not disseminated rash · Patients generally not ill
Drug eruptions	· Exposure to medications · Rash usually maculo-papular, often generalized
Contact dermatitis	· Itching · Contact with possible allergens · Rash often localized in a pattern suggesting external contact
Erythema multiforme minor	· Target, "bull's eye," or iris lesion · Often follows recurrent herpes simplex virus infections · May involve hands and feet (including palms and soles)
Enteroviruses infection, especially Hand, Foot, and Mouth Disease	· Major form involves mucous membranes and conjunctivae · There may be target lesions or vesicles
Disseminated herpes simplex	· Lesions indistinguishable from varicella · Immunocompromised host

Scabies; insect bites (including fleas)	· Itching is a major symptom · Patient is not febrile and is otherwise well · Burrows and rash between fingers not on palms and soles
Molluscum contagiosum	· May disseminate in immunosuppressed persons · Can occur anywhere on the body; usually solitary or few lesions · Presents as small, raised, and usually white, pink, or flesh-coloured lesions with a dimple or pit in the centre

A.3 Diagnostic aspects

Specimen collection, transport, handling and shipping

All specimens collected for laboratory investigations should be regarded as potentially infectious and handled with caution. The recommended specimen types for laboratory confirmation of orthopoxvirusare skin lesions, (swabs of lesion surface and/or exudate, roofs or lesion crusts). Both dry swabs and swabs placed in viral transport media (VTM) could be used. Two lesions of the same type should be collected in one tube, preferably from different locations on the body and which differ in appearance. Lesions, crusts and vesicular fluids should not be mixed in the same tube. In addition to a lesion specimen, the collection of an oropharyngeal swab is recommended. Specimens should be stored refrigerated or frozen within an hour from collection and transported to the laboratory in the shorted possible time. Transport of specimens should comply with any applicable national and/or international regulations, including the UN Model Regulations and any other applicable regulations depending on the mode of transport being used. For international transport, specimens from suspected probable or confirmed cases, including clinical samples, viral isolates and cultures should be transported as Category A, UN2814 "infectious substance, affecting humans". All specimens being transported should have appropriate triple packaging, labelling and documentation. Shipping requires a dangerous goods certified shipper. Correct handling and storage of specimens during transportation is essential for accurate diagnostic testing.

In the event of a suspected smallpox case, effective communication and precautionary measures between the specimen collector and laboratory staff is essential to maximize safety during specimen handling. This is especially relevant in hospital settings, where laboratories routinely process specimens from patients with a variety of infectious and non-infectious conditions.

Further information is available in the WHO Interim guidance on Diagnostic testing for the monkeypox virus (MPXV), May 2024 available at https://iris.who.int/bitstream/handle/10665/376952/WHO-MPX-Laboratory-2024.1-eng.pdf?sequence=1 .

Tests for Detection and Identification of *Orthopoxvirus*

Smallpox

Detection of smallpox can be conducted using:

- molecular test by PCR from vesicular fluid or crust,
- viral isolation (difficult to access),
- demonstration of viral antigens in exudates or crusted materials by complement fixation, immunofluorescence, immunoprecipitation,
- serological tests for the determination of specific antibodies and evidence of seroconversion with an increase of 4-fold or more in antibody titre in subjects not recently vaccinated (cross-reactivity to other orthopoxviruses, e.g. monkeypox, cowpox, should be considered),
- electron microscopy from skin lesion, although not used routinely, in order to visualize the characteristic brick shape of viral particles.

Laboratory criteria for confirmation are the following (Official Journal of the European Union (Commission Implementing Decision (EU) 2018/945):

At least one of the following two laboratory tests:

- Isolation of smallpox (Variola virus) from a clinical specimen followed by sequencing (designated P4 laboratories only)
- Detection of Variola virus nucleic acid in a clinical specimen followed by sequencing Laboratory results need to be interpreted according to the vaccination status

Laboratory criteria for a probable case:

- Identification of orthopox virus particles by EM

Other orthopoxviruses including mpox

Confirmation of MPXV infection is based on nucleic acid amplification testing (NAAT), using real-time or conventional PCR, for detection of unique sequences of viral DNA. PCR can be used alone, or in combination with sequencing. Several groups have developed validated PCR protocols for the detection of OPXV and more specifically MPXV, some of which include distinction clade I and clade II. When the clinical presentation and epidemiology suggest an infection with MPXV despite negative PCR results, serological testing may be useful to further investigate prior infection for epidemiological purposes.

Further information is available in the WHO Interim guidance on Diagnostic testing for the monkeypox virus (MPXV), May 2024 available at https://iris.who.int/bitstream/handle/10665/376952/WHO-MPX-Laboratory-2024.1-eng.pdf?sequence=1.

A.4 Case definition and patient management

Case definition

Smallpox

(Official Journal of the European Union (Commission Implementing Decision (EU) 2018/945)

Clinical Criteria

Any person with at least one of the following two:

- Fever AND Vesicles or firm pustules rash at the same stage of development with a centrifugal distribution
- Atypical presentations defined as at least one of the following four:
    - Haemorrhagic lesions
    - Flat velvety lesions not progressing to vesicles
    - Variola sine eruptione
    - Milder type

Laboratory Criteria

At least one of the following two laboratory tests:

- Isolation of smallpox (Variola virus) from a clinical specimen followed by sequencing (designated P_4 laboratories only)
- Detection of Variola virus nucleic acid in a clinical specimen followed by sequencing Laboratory results need to be interpreted according to the vaccination status

Laboratory criteria for a probable case:

- Identification of orthopox virus particles by EM

Epidemiological Criteria

At least one of the following two epidemiological links:

- Human to human transmission
- Laboratory exposure (where there is a potential exposure to Variola virus)

Case Classification

A. Possible case: Any person meeting the clinical criteria

B. Probable case: Any person meeting the clinical criteria and with at least one of the following two:

- An epidemiological link to a confirmed human case by human to human transmission
- Meeting the laboratory criteria for a probable case

C. Confirmed case: Any person meeting the laboratory criteria for case confirmation

During an outbreak: any person meeting the clinical criteria with an epidemiological link

Mpox

Case definition may vary based on the Clades and on the current outbreaks. UE reporting system (Tessy) has adopted the WHO available at: https://www.who.int/publications/i/item/WHO-MPX-Surveillance-2024.1

Patient Management

Smallpox

Suspected or confirmed cases of smallpox should preferably be managed in high-level isolation units. If such facilities are not available or capacity is overwhelmed, ordinary negative pressure isolation rooms may be used and standard, contact, droplet and respiratory precautions should be implemented. If negative pressure isolation rooms are not available, these patients may need to be isolated in other rooms while implementing the above-mentioned transmission-based precautions. Cohort isolation may be an option, put preferably in a building that does not share ventilation with patients or personnel not caring for smallpox patients.

If transportation is needed, patients should preferably be transported using a negative pressure isolator stretcher equipped with HEPA (High Efficiency Particulate Air) filters. In the absence of such transport devices, the parts of the vehicle or aircraft most exposed to contact with the patient and his excretions must be covered with plastic sheets, in order to facilitate subsequent cleaning and disinfection operations. Ambulance staff should wear appropriate PPE.

After the transport is completed, the vehicle or isolator stretcher must be disinfected according to instructions by the manufacturer of the disinfectant used. It is also important that this disinfection procedure is approved by the owner or operator of the vehicle or isolator stretcher.

Contact tracing should be conducted to identify persons that may have been exposed and the index case. Closely monitor exposed individuals and contacts of clinical cases for at least 17 days following their last contact with confirmed cases. This includes measuring their temperature twice daily and immediately isolating them at the first sign of symptoms. Pre-and post-exposure vaccination prophylaxis should be administered based on a risk assessment.

Mpox

In presence of mild or uncomplicated Mpox and not at high risk of complication, home isolation is reasonable.

Self-isolation includes:

- Stay in a separate room/area away from other household members.
- Avoid share potentially contaminated items, such as bed linens, clothing, towels, wash cloths, drinking glasses or eating utensils.
- Avoid close physical contact, including sexual contact, with others, especially with those at higher risk of severe MPX illness (i.e., people who are immunocompromised and/or pregnant, and children under 8 years of age).
- Avoid contact with animals, including household pets, poultry, and livestock.
- Avoid use of contact lenses to prevent inadvertent infection of the eye.
- Use a separate bathroom if there are others who live in the same household.
- Clean hands often with alcohol-based hand rub or soap and water, including before exiting the place of self-isolation and upon return.

If close contact with others in the home is unavoidable (e.g., the case is a caregiver or receives caregiving support), the case should:

- Wear a medical mask for source control, if safe and tolerated, especially if respiratory symptoms such as a cough or sore throat are present and/or if there are lesions inside the mouth/oral cavity.
- Cover all skin lesions with clothing, bandages, medical mask, and/or gloves.

- If there is not a separate bathroom in the home, the patient should clean and disinfect surfaces such as counters, toilet seats, faucets, using 0.1% sodium hypochlorite solution or alcohol 70.

Patients who need hospitalisation should be placed in an airborne infection isolation single room with a dedicated toilet. If single rooms are not available and multiple MPX cases are present, their cohorting can be considered. If the patient can tolerate it, cover exposed MPX-related lesions if other persons are in the room, including HCWs when not performing a physical exam (ECDC).

Treatment

- *Orthopoxvirus* infections are primarily managed by supportive care, including hydration, nutritional support, and prevention of secondary infections.
- To minimize virus transmission, all skin lesions should be covered until fully healed, and patients should be advised to avoid touching their eyes without washing hands thoroughly.
- Individuals at high risk for severe disease, such as those with weakened immune systems or underlying skin conditions, pose significant management challenges, especially if they develop eye infections.
- Antivirals may be considered. and their use in a bioterrorism scenario would require careful consideration of all affected patients. Antivirals with potential benefit for patients with smallpox and other orthopoxviruses include:
    - Tecovirimat is an antiviral that inhibits VP37 (the product of the F13L gene), a highly conserved protein present in all orthopoxviruses, preventing the formation and egress of enveloped virions.
    - Cidofovir, a nucleoside analogue with broad-spectrum activity against DNA viruses, including poxviruses, mainly used to treat cytomegalovirus retinitis. Dosage 5 mg/kg weekly intravenous. Authorised at national level in EU Member States for other indications than orthopoxviruses.
    - Brincidofovir is a lipid prodrug of cidofovir, which inhibits DNA polymerase and acts as a nucleotide analogue of deoxycytidine monophosphate, which can be incorporated into viral DNA, impeding its synthesis. Not approved by EMA. Dosage 200 mg twice weekly.
- Since smallpox has been eradicated, conducting studies to evaluate the efficacy of antivirals in humans has not been possible. Therefore, efficacy has been demonstrated through in vitro studies and multiple animal models. The combination of tecovirimat with brincidofovir has shown synergistic efficacy against *Orthopoxvirus* infections in vitro and in vivo. The use

of tecovirimat, alone or in combination with vaccines, has been considered for pre-exposure and post-exposure prophylaxis, but there are no data to support its use in these settings.

Vaccination

- First-generation smallpox vaccines played a key role in eradication. However, they were associated with risks of serious adverse events, so new third-generation vaccines have been developed that have an improved safety profile.
- Vaccination can be used both pre-exposure and post-exposure in the population at potential risk.
- Post-exposure vaccination within 4 days, regardless of symptoms, to reduce severity and protect against fatal outcome. There are currently a few vaccines approved by multiple regulatory agencies for the prevention of smallpox, including MVA-BN Modified Vaccinia Ankara Bavarian Nordic approved by EMA with two doses 28 days apart. An additional vaccine is LC16m8, an attenuated, replicating smallpox vaccine derived from the Lister strain of vaccinia, not licensed by EMA.
- Vaccinia immune globulin (VIG) is a hyperimmune globulin prepared for the treatment of certain complications of vaccination with first-generation vaccines. A clear benefit for vaccinia immune globulin in the treatment of smallpox has not been demonstrated, but it may provide cross-protection between orthopoxviruses and has been used in the treatment of smallpox in immunosuppressed patients.

Further information may be found in the "EMA guidance document on the use of medicinal products for treatment and prophylaxis in case of exposure to biological agents used as weapons of terrorism, crime or warfare" EMA/323691/2024 Corr. Available at: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-guidance-use-medicinal-products-treatment-prophylaxis-case-exposure-biological-agents-used-weapons-terrorism-crime-or-warfare_en.pdf

A.5 Key References

- Tucker JB. Scourge: The Once and Future Threat of Smallpox. New York, NY: Atlantic Monthly Press; 2001.
- Fenner F, Henderson DA, Arita I, Jezek A, Ladnyi ID. Smallpox and Its Eradication. Geneva, Switzerland: World Health Organization, 1988.
- 3 AJ. Goff, SC. Johnston, J Kindrachuk, K L. Lin, B Peter, Jahrling, JW. Huggins, M Sofi Ibrahim J. Lawler, JW. Martin. Chapter 24 Smallpox and related Orthopoxviruses. Year 2018; available at https://api.semanticscholar.org/CorpusID:160006343
- Ferguson NM, Keeling MJ, Edmunds WJ, et al. Planning for smallpox outbreaks. Nature. 2003;425:681–685.

- Kaplan EH, Craft DL, Wein LM. Emergency response to a smallpox attack: the case for mass vaccination. Proc Natl Acad Sci U S A. 2002;99:10935–10940.
- Cono J, Casey CG, Bell DM. Smallpox vaccination and adverse reactions: guidance for clinicians. MMWR Recomm Rep. 2003;52:1–28.
- https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/smallpox-and-other-orthopoxvirus-associated-infections. Accessed on 31st May 2024
- https://www.cdc.gov/smallpox/symptoms/index.html. Accessed on 31st May 2024
- https://www.sciencedirect.com/topics/medicine-and-dentistry/orthopoxvirus. Accessed on 31st May 2024
- Billioux, B. J., Mbaya, O. T., Sejvar, J., & Nath, A. (2022). Neurologic complications of smallpox and monkeypox: a review. JAMA neurology, 79(11), 1180-1186.
- Foege WHMillar JDHenderson DA Smallpox eradication in West and Central Africa. Bull World Health Organ. 1975;52209- 222
- Baker AR The eye complications of smallpox: some observations during the recent epidemic in Cleveland. JAMA. 1903;41645- 648
- Impelluso G, Lentzos F. The Threat of Synthetic Smallpox: European Perspectives. Health Secur. 2017 Nov/Dec;15(6):582-586. doi: 10.1089/hs.2017.0045. Epub 2017 Nov 27. PMID: 29178813
- Noyce RS, Lederman S, Evans DH. Construction of an infectious horsepox virus vaccine from chemically synthesized DNA fragments. PLoS One. 2018 Jan 19;13(1):e0188453. doi: 10.1371/journal.pone.0188453. PMID: 29351298; PMCID: PMC5774680.
- https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON493
- Isidro J, Borges V, Pinto M, Sobral D, Santos JD, Nunes A, Mixão V, Ferreira R, Santos D, Duarte S, Vieira L, Borrego MJ, Núncio S, de Carvalho IL, Pelerito A, Cordeiro R, Gomes JP. Phylogenomic characterization and signs of microevolution in the 2022 multi-country outbreak of monkeypox virus. Nat Med. 2022 Aug;28(8):1569-1572.
- Ulaeto DO, Dunning J, Carroll MW. Evolutionary implications of human transmission of monkeypox: the importance of sequencing multiple lesions. Lancet Microbe. 2022 Sep;3(9):e639-e640.
- Zahmatyar M, Fazlollahi A, Motamedi A, Zolfi M, Seyedi F, Nejadghaderi SA, Sullman MJM, Mohammadinasab R, Kolahi AA, Arshi S, Safiri S. Human monkeypox: history, presentations, transmission, epidemiology, diagnosis, treatment, and prevention. Front Med (Lausanne). 2023 Jul 20;10:1157670. Sklenovska N, Van Ranst M. Emergence of monkeypox as the most important orthopoxvirus infection in humans. Front Public Health. (2018) 6:241. 10.3389/fpubh.2018.00241
- Karagoz A, Tombuloglu H, Alsaeed M, Tombuloglu G, AlRubaish A, Mahmoud A, et al. Monkeypox (mpox) virus: classification, origin, transmission, genome organization, antiviral drugs, and molecular diagnosis. J Infect Public Health. (2023) 16:531–41. 10.1016/j.jiph.2023.02.003
- Vaccinia. https://emedicine.medscape.com/article/231773-overview Accessed on 31st May 2024

- https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/smallpox-and-other-orthopoxvirus-associated-infections Accessed on 31st May 2024
- Tropical infectious disease 3rd edition, Edited by: Richard L. Guerrant, David H. Walker and Peter F. Weller
- Breman JG, Henderson DA. Diagnosis and management of smallpox. N Engl J Med. 2002 Apr 25;346(17):1300-8. doi: 10.1056/NEJMra020025. Epub 2002 Mar 28. PMID: 11923491.
- https://www.cdc.gov/smallpox/clinicians/diagnosis-evaluation.html Accessed on 31st May 2024
- Hussain A, Kaler J, Lau G, Maxwell T. Clinical Conundrums: Differentiating Monkeypox From Similarly Presenting Infections. Cureus. 2022 Oct 4;14(10):e29929. doi: 10.7759/cureus.29929. PMID: 36348880; PMCID: PMC9634140.
- WHO. Interim guidance MPXV 23May2022 Accessed on May 31st, 2024
- Ministry Of Health and National Public Health Institute Of Liberia National Technical Guidelines For Integrated Disease Surveillance & Response.
- Meyer H, Ehmann R, Smith GL. Smallpox in the Post-Eradication Era. Viruses. 2020 Jan 24;12(2):138. doi: 10.3390/v12020138. PMID: 31991671; PMCID: PMC7077202.
- Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. J Virol Methods. 2010;169(1):223–7. doi: 10.1016/j.jviromet.2010.07.012. Epub 2010 Jul 17.
- Schroeder K, Nitsche A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. Mol Cell Probes. 2010;24(2):110–3. doi: 10.1016/j.mcp.2009.10.008. Epub 2009 Oct 29.
- Maksyutov RA, Gavrilova EV, Shchelkunov SN. Species-specific differentiation of variola, monkeypox, and varicella-zoster viruses by multiplex real-time PCR assay. J Virol Methods. 2016;236:215–20. doi: 10.1016/j.jviromet.2016.07.024. Epub 2016 Jul 28
- Ropp SL, Jin Q, Knight JC, Massung RF, Esposito JJ. PCR strategy for identification and differentiation of smallpox and other orthopoxviruses. J Clin Microbiol. 1995;33(8):2069–76. doi: 10.1128/jcm.33.8.2069-2076.1995.
- Espy MJ, Cockerill III FR, Meyer RF, Bowen MD, Poland GA, Hadfield TL et al. Detection of smallpox virus DNA by LightCycler PCR. J Clin Microbiol. 2002;40(6):1985–8. doi: 10.1128/JCM.40.6.1985-1988.2002.
- Li D, Wilkins K, McCollum AM, Osadebe L, Kabamba J, Nguete B et al. Evaluation of the GeneXpert for human monkeypox diagnosis. Am J Trop Med Hyg. 2017;96(2):405–10. doi: 10.4269/ajtmh.16-0567.
- Townsend MB, MacNeil A, Reynolds MG, Hughes CM, Olson VA, Damon IK et al. Evaluation of the Tetracore Orthopox BioThreat® antigen detection assay using laboratory grown orthopoxviruses and rash illness clinical specimens. J Virol Methods. 2013;187(1):37–42. doi: 10.1016/j.jviromet.2012.08.023. Epub 2012 Sep 5
- Nakhaie M, Arefinia N, Charostad J, Bashash D, Haji Abdolvahab M, Zarei M. Monkeypox virus diagnosis and laboratory testing. Rev Med Virol. 2023 Jan;33(1):e2404. doi: 10.1002/rmv.2404. Epub 2022 Nov 4. PMID: 36331049.

Biological agents – *Yersinia pestis*

B.1 Introduction

Plague is caused by the bacillus *Yersinia pestis*, a nonmotile, gram-negative coccobacillus belonging to the family *Enterobacteriaceae*. It evolved several thousand years ago from *Y. pseudotuberculosis*. *Y. pestis* is an obligate pathogen, meaning it relies on a host organism to complete its life cycle and cannot survive long-term outside of a host. Three phenotypically different biovars have been identified, and molecular data indicate that they correspond to phylogenetically distinct groups. The bacterium harbours a plasmid that produces toxins, contributing to its high lethality.

Plague is endemic in many countries and regions around the world. Rodents are animal reservoirs with flees acting as vectors. While eradicating *Y. pestis* in nature is not feasible, effective mitigation strategies, such as controlling flea vectors, can significantly reduce its impact.

Y. pestis and its potential for intentional release.

Y. pestis is recognised as a potential bioterrorism agent due to several properties:

- High case fatality, especially pneumonic plague, if not treated promptly.
- Ease of dissemination: *Y. pestis* can be aerosolized, making it possible to spread through the air and infect large populations quickly.
- Historical use: There are historical instances of *Y. pestis* being used as a biological weapon.
- Public panic: The severe symptoms and high mortality rate can cause significant public fear and social disruption.
- Accessibility. *Y. pestis* is endemic in many countries, due to existing natural foci.

Some elements can help in differentiating a natural from an intentional event:

- Identification of *Y. pestis* infections in non-endemic areas,
- unusual patient (e.g. not exposed to animal, vector, symptomatic patient),
- unusual agent (genetically engineered) or microbiological features (e.g. virulence factor, antibiotic resistance, unexpected virulence features, including F1 -negative strain, signs of genetic engineering of *Y. pestis*),
- unusual features of outbreak (e.g. very sharp increase, occurring in place not endemic for plague, occurring in unusual population, several different unrelated outbreaks).

Potential methods of deliberate release

The following main routes of transmission can be considered for deliberate release of *Y. pestis*:

- aerosolisation, causing primary plague pneumonia,
- contamination of the food or water supply. Less likely but could lead to primary pharyngeal, bubonic, or septicaemic plague,
- introduction of infected animals or vectors (e.g. fleas).

Aerosolization is considered a high consequence scenario. Aerosol dissemination can occur by liquid spraying, or by dry powder release. Aerosolized biological warfare agents, in particulate form between 0.3–5 µm in diameter pose a huge risk to the public in a bioweapons attack. In 1970, The WHO estimated that if 50 kg of *Y. pestis* were released as an aerosol over a city of 5 million, pneumonic plague could occur in as many as 150 000 persons, 36 000 of whom would be expected to die. The plague bacilli would remain viable as an aerosol for 1 hour for a distance of up to 10 km.

The appearance of plague-infected vectors (fleas) or animal reservoirs (rodents) outside known endemic areas, along with increased mortality rates among susceptible animal populations and unexpected epizootic spread, could indicate an intentional release.

Potential Secondary Spread in case of intentional release

After the initial intentional release of *Y. pestis*, secondary spread may occur by

- Re-aerosolization: This can happen when an aerosol deposited onto various surfaces or environmental media (e.g., soil, water) becomes airborne again.
- Person-to-person transmission: Infectious respiratory droplets from coughing, talking, and breathing can spread pneumonic plague.
- Spillover from animal reservoir:
    - Enzootic spread: Vector (flea) and animal reservoir (primarily rodents) control is required to mitigate the potential for *Y. pestis* transmission among non-human hosts, especially in areas with natural enzootic cycles of infection.
    - Epizootic spread: Infected rodents or fleas can cause epizootic outbreaks of *Y. pestis* among other animals, which could facilitate plague spread following an intentional aerosol release. Infected non-human hosts (including domestic pets, such as cats and dogs) may also transmit plague to humans. Most human plague cases are associated with epizootic, rather than enzootic transmission.

B.2 Clinical Presentations and differential diagnosis

Clinical presentation of *Y. pestis*

Humans are accidental hosts for *Y. pestis* and are highly susceptible to the infection. The clinical form depends on the route of the infection. In humans, *Y. pestis* infection has three main clinical manifestations: bubonic plague, which is most common after natural infection, septicaemic plague, and pneumonic plague. The latter is expected to be the most common form following an intentional event. Less common forms are pharyngeal and meningeal plague.

Bubonic plague

Bubonic plague is the most common form in humans and accounts for 80-95% of all cases worldwide. The incubation period is of 1–6 days. Bubonic plague can be acquired by

- Flea Bites: The most common way is through the bite of an infected flea, which has fed on a rodent carrying *Y. pestis*.
- Direct Contact: Scratches or bites from infected domestic cats or other infected animals. Handling tissues or bodily fluids from an infected animal can also transmit the bacteria through broken skin.

After exposure, the bacteria spread rapidly from the inoculation site via lymphatics to the nearest regional lymph nodes. Patients develop flu-like symptoms in the early stage of the infection, characterised by sudden onset of high-grade fever, headache, chills, and severe lethargy. Simultaneously, patients develop extremely painful swollen lymph nodes called buboes. The femoral and inguinal lymph nodes are most commonly affected in adults, followed by the axillary and cervical nodes. In children, upper body sites may be relatively more involved. The lymphatic tissue becomes oedematous, and the overlying skin may be erythematous, warm, and tense, and may desquamate because of infiltration and necrotisation. The infected lymph nodes are severely damaged, and the pathological picture is characterised by haemorrhage and necrosis. Without prompt and adequate therapy, bubonic plague mortality rates reach 60%. However, bubonic plague responds well to antibiotic treatment, and mortality rates with proper treatment is below 5%.

Bubonic plague is not transmissible between persons.

Pneumonic form

Pneumonic plague can spread from person to person and lead to localized outbreaks, or even devastating epidemics. The infectious dose by inhalation can be as low as 100–500 organisms. Primary pneumonia results from inhalation of infectious aerosols and has a short incubation period of 1–3 days. Secondary pneumonic plague can occur via haematogenic spread of bubonic or septicaemic forms. Untreated, pneumonic plague is usually fatal within 2–4 days after respiratory

exposure; in some instances, death from primary plague pneumonia occurs as rapidly as 15–24 hours after exposure.

The onset of primary pneumonic plague is sudden with unspecific symptoms of high-grade fever, headache, chills, weakness, dizziness, and chest pain. Cough, sputum production, rapidly increasing chest pain, tachypnoea and dyspnoea typically predominate on the second day of illness. However, some patients may develop serious symptoms within a few hours of onset, including haemoptysis, multiorgan failure, respiratory distress, cardiopulmonary failure, cyanosis, and circulatory collapse. Primary pneumonic pneumonia, essentially an alveolar process, is characterised by sputum production and is initially watery or mucoid, but rapidly becoming bloody. At the site of consolidation, liquefactive necrosis and cavitation may develop, leaving significant residual scarring.

Secondary pneumonic plague is a consequence of haematogenic spread as it occurs when the bacteria enter the bloodstream and spreads to the lungs.

Septicaemic form

The septicaemic form of plague is a serious, progressive bloodstream infection resulting from the haematogenous spread of *Y. pestis*. Patients may have gastrointestinal symptoms as well, such as abdominal pain, diarrhoea, nausea, and vomiting. Primary septicaemic plague occurs when *Y. pestis* directly enters the bloodstream, typically through the bite of an infected flea or direct contact with infected tissues. Secondary septicaemic plague develops as a complication of bubonic or pneumonic plague, where the bacteria spread from the initial site of infection (such as the lymph nodes or lungs) into the bloodstream.

Without prompt and adequate therapy using appropriate antibiotics and aggressive supportive care, septicaemic plague is usually fatal. Some patients develop acute respiratory distress syndrome (ARDS).

Meningeal Plague

Meningitis is a rare manifestation of plague and is a complication of bubonic plague, or the other forms of plague. Although meningitis may be a part of the initial presentation of plague, the onset is often delayed and may be the result of inadequate antibiotic treatment of the primary illness. Plague meningitis symptoms are similar to typical bacterial meningitis, with fever, headache, altered mental status, nuchal rigidity, meningismus, and a polymorphonuclear leukocytic pleocytosis.

Pharyngeal Plague

Plague pharyngitis is relatively rare and presents with symptoms such as fever, sore throat, and cervical lymphadenitis. In the early stage, it may be clinically indistinguishable from other common causes of pharyngitis. Cervical or submandibular buboes usually develop secondary to the pharyngeal involvement. Pharyngeal plague can develop after respiratory exposure or consumption

of undercooked meat infected with *Y. pestis*. This form of plague increases the likelihood of secondary plague pneumonia.

Table 1. Characteristics of different clinical forms of Yersinia pestis infection

Clinical form	Incubation period	Main symptoms
Bubonic plague	1-6 days	flu-like symptoms, high-grade fever, swollen lymph nodes, and sudden onset of painful lymphadenitis
Septicaemic plague	1-6 days	high-grade fever, chills, multiorgan failure
Pneumonic plague	1-6 days	high-grade fever, cough, haemoptysis, multiorgan failure, respiratory distress, cardiopulmonary failure, cyanosis and circulatory collapse
Pharyngeal plague	1-6 days	fever, sore throat, and cervical lymphadenitis
Meningeal plague	1-6 days	fever, headache, altered mental status, nuchal rigidity, meningismus, and a polymorphonuclear leukocytic pleocytosis

Differential diagnosis

Differential diagnosis is very important as many diseases can cause similar symptoms in the different stages. Prompt and adequate therapy should be provided on suspicion before microbiological confirmation.

Table 2. Differential diagnosis of bubonic plague from other pathogens

Infection/disease	Characteristics
Staphylococcus aureus, and *Streptococcus pyogenes* caused adenitis	• Purulent or inflamed lesion often noted distal to involved nodes (i.e., pustule, infected traumatic lesion) • Involved nodes more likely to be fluctuant • Associated ascending lymphangitis or cellulitis may be present (generally not seen with plague)
Infectious mononucleosis	• Sore throat, fever, swollen glands, and tiredness or fatigue.

Francisella tularensis	• Ulcer or pustule often present distal to involved nodes • Clinical course rarely as fulminant as in plague • Systemic toxicity uncommon
Bacillus anthracis	• Painless lymphadenitis, eschar
Bartonella henselae (cats scratch disease)	• History of contact with cats; usually history of cat scratch • Incubation period: 3-14 days • Primary lesion at the site of scratch • Small papule and vesicle • Late lymphoadenopathy
Bartonella quintana (trench fever)	• *B. quintana* is transmitted by the human body louse, *P. humanus corporis* • The infection is associated with poverty, lack of hygiene, and cold weather • Incubation period typically varies from 15 to 25 days, down to 6 days • Persistent bacteraemia, endocarditis, lymphadenopathy
Primary or secondary syphilis (*Treponema pallidum*)	• Enlarged lymph nodes in the inguinal region • Lymph nodes generally are painless • Chancre may be noted with primary syphilis and painless
Chancroid (*Haemophilus ducreyi*)	• Sexually transmitted infection • Most prevalent genital ulcerative disease in Africa • Incubation period: 2-10 days or up to 14 days • Adenitis occurs in the inguinal region • Ulcerative lesion present • Systemic symptoms uncommon; toxicity does not occur
Primary genital herpes	• Lesions present in genital area • Adenitis occurs in the inguinal region
Mycobacterial infection, scrofula (Mycobacterium tuberculosis and other related *Mycobacterium* species)	• Adenitis occurs in cervical region • Usually painless • Indolent clinical course

Lymphatic filariasis	• Heavy microfilarial loads may develop acute and chronic inflammatory granulomas secondary to splenic destruction
Rickettsial diseases	• Vary considerably in severity from • Self-limiting mild illnesses to severe life-threatening infections
Chlamydia trachomatis (lymphogranuloma venereum) and *Calymmatobacterium granulomatis* (donovanosis)	• Sexually transmitted infection • Incubation period: 10-30 days • Adenitis occurs in the inguinal region • Suppuration, fistula tracts common • Although LGV buboes may be somewhat tender, exquisite tenderness usually absent Although patients may appear ill (headache, fever, myalgias), systemic toxicity not present

Table 3. Differential diagnosis of septicaemic plague from other pathogens:

Typhoid fever	laboratory diagnosis
Other bacterial sepsis	laboratory diagnosis
Non-specific sepsis syndrome	laboratory diagnosis
Malaria	symptoms within 10-15 days after bite of Mosquito

Table 4. Differential diagnosis of pulmonary plague from other pathogens:

Inhalational anthrax (*Bacillus anthracis*)	• Widened mediastinum and pleural effusions seen on CXR or chest CT • Minimal sputum production • Haemoptysis is uncommon (if present, suggests diagnosis of plague)
Tularaemia (*Francisella tularensis*)	• Not as rapid or fulminant as in pneumonic plague
Mycoplasma pneumoniae and pneumonia caused by *Chlamydia pneumoniae*	• Rarely as fulminant as pneumonic plague • Walking pneumonia in *Mycoplasma pneumoniae* • Minimal sputum production
Streptococcal pneumonia caused by *Streptococcus pneumoniae*	• Rarely as fulminant as pneumonic plague • Rapid tests available - POC diagnosis • Sputum production is similar, haemoptysis is frequent
Staphylococcal pneumonia	• Rarely as fulminant as pneumonic plague • Seasonal outbreaks are common
Legionnaires' disease (*Legionella pneumophila* or other Legionella species	• Rarely as fulminant as pneumonic plague • Community outbreaks of Legionnaires' disease often connect to exposure of cooling systems, water systems
Psittacosis (*Chlamydia psittaci*)	• Rarely as fulminant as pneumonic plague • Result of bird stool exposure
Influenza	• Influenza generally seasonal (October-March-April in Europe and USA) or involves history of recent cruise ship travel or travel to tropics • Highly pathogenic Influenza viruses travel history and exposure of infected birds, cows, etc.
Hantavirus pulmonary syndrome	• Exposure of urine or faeces of small rodents, in majority mice with Hantavirus • Travel to the Americas
Respiratory Syncytial Virus (RSV)	• RSV usually occurs in children or elderly patients • Shows seasonality (winter/spring)
Q fever (*Coxiella burnetii*)	• Exposure to infected parturient cats, cattle, sheep, goats • Severe pneumonia not prominent feature
MERS	• Travel history •
SARS-CoV-2	• Broad clinical spectrum • Severe pneumonia case

B.3 Diagnostic aspects

Specimen collection, transport, handling, and shipping

Specimen collection

The recommended specimen types for laboratory confirmation of *Y. pestis* are the following:

Bubonic plague:

- pus,
- lymphoid tissue, biopsy (core or aspiration),
- blood, anti-coagulated blood (EDTA blood),
- isolates of gram-negative bacilli, or identified previously as *Yersinia pestis.*

Septicaemic and pneumonic, pharyngeal, meningeal plague:

- respiratory samples: sputum, BAL, tracheal aspirate,
- blood, anti-coagulated blood (EDTA blood), blood culture,
- urine,
- stool,
- cerebrospinal fluid, depends on the form and the stage of the disease,
- post-mortem specimen of lymphoid tissues, lung, brain and bone marrow,
- isolates of gram-negative bacilli or identified previously as *Yersinia pestis.*

Instructions for sample collection:

- Specimen collection from plague patients should be performed using personal protective equipment.
- Complete the history form, including clinical information, travel history, animal contact, personal close contacts, etc. to insure optimal processing of the specimen.
- Carefully label all sample collection tubes.
- Disposable materials should be used for sample collection.
- If deliberate release is suspected, law enforcement authorities should be consulted regarding forensic considerations during sampling.

Transportation of specimens

- Specimens collected for *Y. pestis* investigation should be refrigerated (2 - 8 °C) or frozen within one hour after collection.
- If transport exceeds 7 days for the sample to be tested, specimens should be stored at -20 °C or lower. Longer-term specimen storage (>60 days from collection) is recommended at -20°C. Repeated freeze-thaw cycles should be avoided because they can reduce the quality of specimens.

- Transport of specimens should comply with any applicable national and/or international regulations, including IATA (www.iata.org).
- For national and international transport, specimens from suspected probable or confirmed cases of *Y. pestis*, including clinical samples, isolates should be transported as Category A, UN2814 "infectious substance, affecting humans".
- According to P620 package all specimens and isolates being transported should have appropriate triple packaging, labelling and documentation. Shipping requires a dangerous goods certified shipper. Correct handling and storage of specimens during transportation is essential for accurate diagnostic testing.
- All the samples collected for routine testing and cultures should be handled in Biosafety Level 2 (BSL-2) laboratories. Large-scale cultures and activities with high potential for droplet or aerosol production (centrifuging, grinding, etc.) require Biosafety Level 3.
- If a genetically modified pathogen is suspected, additional risk assessment should be performed before the laboratory work and, if necessary, elevated biosafety level should be used.
- Trained laboratory personnel processing and conducting analyses of samples for *Y. pestis* must use appropriate PPE (e.g. respiratory protection, etc.).
- Disposable materials (e.g. pipettes, loops) should be used for sample manipulations.

Tests for Detection and Identification of *Yersinia pestis*

The laboratory criteria for diagnosis, as recommended by the ECDC, CDC, and WHO, include:

- Isolation of *Y. pestis* from a clinical specimen with culture identification. Validation by a secondary assay is necessary (e.g., PCR, bacteriophage lysis assay, direct fluorescent antibody assay) as performed by an accredited laboratory. Compared to many other bacteria, *Y. pestis* grows slowly in the laboratory, with a generation time of 1.25 hours. Slow growth can lead to delayed laboratory confirmation, potentially resulting in severe or fatal consequences for the patient.

- Detection of *Yersinia pestis* specific DNA or antigens, including F1 antigen, in a clinical specimen by direct fluorescent antibody assay (DFA), immunohistochemical assay (IHC), or polymerase chain reaction (PCR).

- Fourfold or greater change in paired serum antibody titre to *Y. pestis* F1 antigen. This may be issued as a preliminary result if elevated serum antibody titre(s) to *Yersinia pestis* fraction 1 (F1) antigen (without documented fourfold or greater change) in a patient with no history of plague vaccination, or previous infection.

An initial positive result must be confirmed by a National Reference Laboratory as soon as possible. All *Y. pestis* clinical specimens or isolates could be send to EU Reference Laboratory for public health on high risk, emerging and zoonotic bacterial pathogens as soon as possible. The designation of European Reference Laboratory based on the Regulation (EU) 2022/2371 of the European Parliament.

B.4 Case definition and patient management

Case definition

(Official Journal of the European Union (Commission Implementing Decision (EU) 2018/945)

Clinical Criteria

Any person with at least one of the following clinical forms:

Bubonic plague:

- Fever AND sudden onset of painful lymphadenitis

Septicaemic plague:

- Fever

Pneumonic plague:

- Fever AND At least one of the following three: Cough; Chest pain; Haemoptysis

Laboratory Criteria

At least one of the following three:

- Isolation of Yersinia pestis from a clinical specimen
- Detection of Yersinia pestis nucleic acid from a clinical specimen
- Yersinia pestis anti-F1 antigen specific antibody response

Epidemiological Criteria

At least one of the following four epidemiological links:

- Human to human transmission
- Animal to human transmission
- Laboratory exposure (where there is a potential exposure to plague)
- Exposure to a common source

Case Classification

A. Possible case: Not applicable

B. Probable case: Any person meeting the clinical criteria with an epidemiological link

C. Confirmed case: Any person meeting the laboratory criteria

Patient management

Bubonic plague patients without secondary pneumonia have a very low risk of spreading plague to close contacts. However, a patient with secondary or primary pneumonic plague can transmit *Y. pestis* to close contacts through respiratory droplets when coughing. Within the first 24h from the infection, the patient develops fever and tachycardia without coughing or haemoptysis. During this period, the patient is non-infectious. In contrast, pneumonic plague patients who cough or expectorates bloody sputum are considered highly infectious.

The main infection prevention and control recommendation are the following (Guidance for healthcare workers on the use of personal protective equipment in the management of bubonic and pneumonic plague patients (europa.eu):

- Strict hospital isolation in a single room with standard and droplet precautions for at least 48 hours from the start of adequate antibiotic therapy with clinical improvement; precautions for drainage and secretions continuous disinfection of excretions and biological fluids and of all materials that have been in contact with the patient, including instruments and laboratory material; If isolation is not possible, they should ensure a separation of at least two metres between patients, and a dedicated bathroom for the infected patient.
- Airborne precautions are typically not necessary but are reasonable during aerosol-generating procedures in patients with pneumonic plague.
- Personal Protective equipment: HCW should wear the following PPE when managing or caring for a patient with possible, probable or confirmed pneumonic plague: gloves ; long-sleeved gown with tight cuffs (single use/disposable preferable) ; eye protection (face shield or goggles) ; respiratory protection (FFP3 filter mask or N-95 particulate).The patient should wear a surgical mask if it is necessary to move him/her around the hospital; HCW should inform the patient about cough etiquette and respiratory hygiene.
- follow the interim guidance on 'How to safely collect sputum samples from patients suspected to be infected with pneumonic plague' for the specimens' collection in case of suspected cases of pneumonic plague (available at: http://www.who.int/csr/disease/plague/collecting-sputumsamples.PDF?ua=1).
- disinfection with approved disinfectants according to national recommendations and active against fleas: disinfection of clothing, personal effects, and patient's luggage.

- Patient transport should be limited to essential purposes only; if the patient is transported out of the room, precautions should be maintained.

- Non-critical patient-care equipment should be dedicated whenever possible. If equipment cannot be dedicated, then it should be adequately cleaned and disinfected between patients.
- In crisis settings where personal protective equipment is not available, pre-exposure prophylaxis with antimicrobials may be reasonable for health care providers and first responders if supplies are sufficient.
- Contact and droplet precautions should be implemented when buboes are being aspirated or irrigated, due to the propensity for aerosolization of infectious material (World Health Organization. How to safely collect pus samples from buboes of patients suspected to be infected with bubonic plague 2016 [cited 2017 Oct 19]. Available from: http://www.who.int/csr/disease/plague/collecting-pussamples.PDF?ua=1).

In general, environmental decontamination following an aerosol event has not been recommended, since it has been estimated that an aerosol of *Y. pestis* organisms would be infectious for only about 1 hour.

Treatment

- Supportive therapy should be administered.
- The keys to the successful treatment of plague are early recognition and timely administration of effective antibiotics.
- The use of other therapies has been reported, including immunotherapy, phage therapy, bacteriocin therapy, and application of virulence factor inhibitors; however, they are not routinely used in clinics.
- Contact tracing should be started as soon as possible, and close contacts should be considered for post-exposure prophylaxis (see below).

Based on cumulative evidence, guidelines for treating plague have recently been updated. Streptomycin, once the preferred antibiotic, is no longer available in most countries and it has been replaced by gentamicin in guidelines. Today, quinolones are also considered first line alternatives that may be used in monotherapy or in combination with gentamicin in severe cases, or when antibiotic resistance is suspected. Initial treatment for patients with severe pneumonic or septicaemic plague, should involve dual therapy with two different classes of antimicrobials.

Naturally occurring antimicrobial resistance in *Y. pestis* is rare, but there is potential for engineered resistance in the context of bioterrorism. Starting treatment with two different classes of antimicrobials increases the chance that the patient will receive at least one effective agent. In the event

of a large-scale plague bioterrorism attack, when numerous cases arise, and parenteral therapy is impractical, oral therapy may be the preferred option.

Alternative antibiotics for treatment of plague include tetracyclines, trimethoprim-sulfamethoxazole, and chloramphenicol. Antibiotic treatment should be administered for 10 to 14 days; clinical improvement is usually evident 2 to 3 days after the beginning of antibiotic treatment, although fever may persist longer.

Postexposure prophylaxis is recommended for individuals who have had close (within 6 feet), prolonged contact with a pneumonic plague patient without adequate personal protective equipment. It is also advisable for laboratory workers accidentally exposed to infectious materials and for those who had close (within 6 feet) or direct contact with infected animals, including veterinary staff, pet owners, and hunters. The prophylaxis should be administered for a duration of 7 days. Quinolones and doxycycline are considered first-line antibiotics for post-exposure prophylaxis of plague.

For the detailed antibiotic administration protocol, refer to the "EMA guidance document on the use of medicinal products for treatment and prophylaxis in case of exposure to biological agents used as weapons of terrorism, crime or warfare" (EMA/323691/2024 Corr. Available at: https://www.ema.europa.eu/en/documents/regulatory-procedural-guideline/ema-guidance-use-medicinal-products-treatment-prophylaxis-case-exposure-biological-agents-used-weapons-terrorism-crime-or-warfare_en.pdf. See also: Nelson CA, Meaney-Delman D, Fleck-Derderian S, Cooley KM, Yu PA, Mead PS. Antimicrobial Treatment and Prophylaxis of Plague: Recommendations for Naturally Acquired Infections and Bioterrorism Response. MMWR Recomm Rep 2021;70(No. RR-3):1–27. DOI: http://dx.doi.org/10.15585/mmwr.rr7003a1 and Antimicrobial Treatment and Prophylaxis of Plague: Recommendations for Naturally Acquired Infections and Bioterrorism Response | MMWR (cdc.gov))

Vaccines

There are currently no approved vaccines against plague. A live *Y. pestis* EV vaccine, previously used with benefit in Madagascar, is used in Asia and Russia, but was never licensed in the EU. Several vaccines candidates are currently under pre-clinical and clinical development.

B.5 Key References

- Ansari, I., Grier, G., & Byers, M. (2020). Deliberate release: Plague - A review. Journal of biosafety and biosecurity, 2(1), 10–22. https://doi.org/10.1016/j.jobb.2020.02.001
- Pechous, R. D., Sivaraman, V., Stasulli, N. M., & Goldman, W. E. (2016). Pneumonic Plague: The Darker Side of *Yersinia pestis*. Trends in microbiology, 24(3), 190–197. https://doi.org/10.1016/j.tim.2015.11.008

- Yang, R., Atkinson, S., Chen, Z., Cui, Y., Du, Z., Han, Y., Sebbane, F., Slavin, P., Song, Y., Yan, Y., Wu, Y., Xu, L., Zhang, C., Zhang, Y., Hinnebusch, B. J., Stenseth, N. C., & Motin, V. L. (2023). *Yersinia pestis* and Plague: some knowns and unknowns. Zoonoses (Burlington, Mass.), 3(1), 5. https://doi.org/10.15212/zoonoses-2022-0040
- Prentice, M. B., & Rahalison, L. (2007). Plague. Lancet (London, England), 369(9568), 1196–1207. https://doi.org/10.1016/S0140-6736(07)60566-2
- Mead P. S. (2011). Plague. Tropical Infectious Diseases: Principles, Pathogens and Practice, 276–283. https://doi.org/10.1016/B978-0-7020-3935-5.00041-0
- Deng, W., Burland, V., Plunkett, G., 3rd, Boutin, A., Mayhew, G. F., Liss, P., Perna, N. T., Rose, D. J., Mau, B., Zhou, S., Schwartz, D. C., Fetherston, J. D., Lindler, L. E., Brubaker, R. R., Plano, G. V., Straley, S. C., McDonough, K. A., Nilles, M. L., Matson, J. S., Blattner, F. R., ... Perry, R. D. (2002). Genome sequence of *Yersinia pestis* KIM. Journal of bacteriology, 184(16), 4601–4611. https://doi.org/10.1128/JB.184.16.4601-4611.2002
- Butler T. (1983). Plague and Other Yersinia Infections. New York: Plenum Medical Book Company; 10.1073/pnas.0603456103
- Flexner S. (1901). The pathology of bubonic plague. Am. J. Med. Sci. 122, 396–416 10.1056/NEJM199709043371004 [CrossRef] [Google Scholar]
- Dennis D. T. (2005). "Plague as a biological weapon," in Bioterrorism and Infectious Disease: A New Dilemma for the 21st Century, eds Fong I. W., Alibek K. (New York: Springer;), 37–70 10.1016/j.chom.2008.09.004
- Pollitzer R. Plague. Geneva: World Health Organization; 1954. p. 233–50.
- Franz DR, Jahrling PB, Friedlander AM, McClain DJ, Hoover DL, Bryne WR, et al. Clinical recognition and management of patients exposed to biological warfare agents. JAMA. 1997;278:399–411. 10.1001/jama.278.5.399
- Evans C. Pneumonic Plague: Incidence, Transmissibility and Future Risks. Hygiene. 2022; 2(1):14-27. https://doi.org/10.3390/hygiene2010002;
- Jacob L. Kool, Robert A. Weinstein, Risk of Person-to-Person Transmission of Pneumonic Plague, Clinical Infectious Diseases, Volume 40, Issue 8, 15 April 2005, Pages 1166–1172, https://doi.org/10.1086/428617
- Cooley, K. M., Fleck-Derderian, S., McCormick, D. W., & Nelson, C. A. (2023). Plague Meningitis: A Systematic Review of Clinical Course, Antimicrobial Treatment, and Outcomes. Health security, 21(1), 22–33. https://doi.org/10.1089/hs.2022.0081;
- Cooley,K., Fleck-Derderian, S., Nelson, C. 104. Plague Meningitis - A Systematic Review of Published Cases, Antimicrobial Treatment, and Outcomes, Open Forum Infectious Diseases, Volume 8, Issue Supplement_1, November 2021, Page S65, https://doi.org/10.1093/ofid/ofab466.104

- Arbaji, A., Kharabsheh, S., Al-Azab, S., Al-Kayed, M., Amr, Z. S., Abu Baker, M., & Chu, M. C. (2005). A 12-case outbreak of pharyngeal plague following the consumption of camel meat, in north-eastern Jordan. Annals of tropical medicine and parasitology, 99(8), 789–793. https://doi.org/10.1179/136485905X65161
- Siengsanan-Lamont, J., & Blacksell, S. D. (2018). A Review of Laboratory-Acquired Infections in the Asia-Pacific: Understanding Risk and the Need for Improved Biosafety for Veterinary and Zoonotic Diseases. Tropical medicine and infectious disease, 3(2), 36. https://doi.org/10.3390/tropicalmed3020036
- Wurtz, N., Papa, A., Hukić, M., Caro, A.D., Leparc-Goffart, I., Leroy, E.M., Landini, M.P., Sekeyová, Z., Dumler, J.S., Bădescu, D., Busquets, N., Calistri, A., Parolin, C., Palù, G., Christova, I., Maurin, M., Scola, B.L., & Raoult, D. (2016). Survey of laboratory-acquired infections around the world in biosafety level 3 and 4 laboratories. European Journal of Clinical Microbiology & Infectious Diseases, 35, 1247 - 1258.
- Regulation (EU) 2022/2370 of the European Parliament and of the Council of 23 November 2022 amending Regulation (EC) No 851/2004 establishing a European centre for disease prevention and control; https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32022R2370
- Regulation (EU) 2022/2371 of the European Parliament and of the Council of 23 November 2022 on serious cross-border threats to health and repealing Decision No 1082/2013/EU; https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32022R2371
- ECDC Disease and laboratory networks; https://www.ecdc.europa.eu/en/about-ecdc/what-we-do/partners-and-networks/disease-and-laboratory-networks
- EU4Health 2024 Work Programme; https://health.ec.europa.eu/publications/2024-eu4health-work-programme_en
- Anisimov AP, Amoako KK. 2006. Treatment of plague: promising alternatives to antibiotics. J Med Microbiol 55:1461–1475. doi: 10.1099/jmm.0.46697-0)
- CDC. 2020. Plague: resources for clinicians. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/plague/healthcare/ clinicians.html).
- Boulanger LL, Ettestad P, Fogarty JD, Dennis DT, Romig D, Mertz G. 2004. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in New Mexico, 1985–1999. Clin Infect Dis 38: 663– 669. https://doi.org/10.1086/381545.;
- CDC. 2020. Plague: resources for clinicians. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/plague/healthcare/ clinicians.html
- Inglesby TV, Dennis DT, Henderson DA, et al, for the Working Group on Civilian Biodefense. Plague as a biological weapon: medical and public health management. JAMA. 2000;283:2281-2290

- Guiyoule A, Gerbaud G, Buchrieser C, Galimand M, Rahalison L, Chanteau S, Courvalin P, Carniel E. 2001. Transferable plasmid-mediated resistance to streptomycin in a clinical isolate of *Yersinia pestis*. Emerg Infect Dis 7:43– 48. https://doi.org/10.3201/eid0701.010106).
- Galimand M, Carniel E, Courvalin P. 2006. Resistance of *Yersinia pestis* to antimicrobial agents. Antimicrob Agents Chemother 50:3233–3236. https://doi.org/10.1128/AAC.00306-06.).
- Lei, C., & Kumar, S. (2022). *Yersinia pestis* antibiotic resistance: a systematic review. Osong public health and research perspectives, 13(1), 24–36. https://doi.org/10.24171/j.phrp.2021.0288
- (Ryzhko IV, Shcherbaniuk AI, Samokhodkina ED, Tsuraeva RI, Mishn'kin Bn Kasatkina IV, Zhigalova TA. 1994. Virulence of rifampicin and quinolone resistant mutants of strains of plague microbe with Fra and Fra phenotypes. Antibiot Khimioter 39:32–36).
- Lei, C., & Kumar, S. (2022). *Yersinia pestis* antibiotic resistance: a systematic review. Osong public health and research perspectives, 13(1), 24–36. https://doi.org/10.24171/j.phrp.2021.0288
- Nishiura H, Schwehm M, Kakehashi M, Eichner M. 2006. Transmission potential of primary pneumonic plague: time inhomogeneous evaluation based on historical documents of the transmission network. J Epidemiol Community Health 60:640 – 645. https://doi.org/10.1136/jech .2005.042424.).
- Kman NE, Nelson RN. 2008. Infectious agents of bioterrorism: a review for emergency physicians. Emerg Med Clin North Am 26:517–547. https://doi.org/10.1016/j.emc.2008.01.006;
- Darling RG, Catlett CL, Huebner KD, Jarrett DG. 2002. Threats in bioterrorism I: CDC category A agents. Emerg Med Clin North Am 20: 273–309. https://doi.org/10.1016/S0733-8627(02)00005-6; Yang R. Plague: Recognition, Treatment, and Prevention. J Clin Microbiol. 2017 Dec 26;56(1):e01519-17. doi: 10.1128/JCM.01519-17. PMID: 29070654; PMCID: PMC5744195.)
- https://www.cdc.gov/infectioncontrol/pdf/guidelines/isolation-guidelines-H.pdf;
- Inglesby TV, Dennis DT, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Fine AD, Friedlander AM, Hauer J, Koerner JF, Layton M, McDade J, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Schoch-Spana M, Tonat K. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. JAMA. 2000 May 3;283(17):2281-90. doi: 10.1001/jama.283.17.2281.

Chemical Agents – Chlorine

C.1 Introduction

This part of the document summarises the immediate and long-term health impact and clinical management for people exposed to chlorine. It should be used alongside any existing national advice and guidelines.

C.2 Properties of chlorine

- Chlorine gas (Cl_2) is a yellowish-green gas at room temperature.
- It has a pungent bleach odour but with chronic exposure people may only be able to detect high concentrations.
- It readily dissolves in water.
- It is corrosive and a strong oxidant.
- It is non-combustible but enhances combustion of other substances.
- It is denser than air so remains at ground level, accumulating in low-lying areas.
- It may combine with water or steam to produce toxic and corrosive fumes of hydrochloric acid.
- It can be stored and transported as compressed gas and in aqueous solutions.

C.3 Health impact of chlorine exposure

Chlorine damages exposed mucous membranes; severity of the injury is dependent on the concentration in the air, duration of exposure and any pre-existing cardio-pulmonary disease.

Chlorine has an odour detection threshold of 0.2-0.4 ppm. Some tolerance to the odour may develop over time. Concentrations of up to 3 ppm may lead to mild mucous membrane irritation and can usually be tolerated for up to an hour, though patients with pre-existing health problems may be susceptible to toxicity at lower concentrations. Concentrations of >3 ppm will be extremely irritating to the eyes and respiratory tract, see Table 2 for signs and symptoms of chlorine exposure. At 1000 ppm, chlorine exposure is fatal within minutes (see Table 1 below for effects at different concentrations). As chlorine is denser than air, confined environments must be considered in the risk assessment. Children and the elderly may experience more severe toxicity which must be considered in the risk assessment.

Table 1. Acute effects of chlorine on the respiratory tract of humans

Concentration (ppm)	Effects

0,2-0.4	**Odour detection**
1-3	Mild mucous membrane irritation
5-15	Moderate irritation
30	Immediate chest pain, dyspnoea, cough
40-60	Chemical pneumonitis and pulmonary oedema
430	Lethal over 30 minutes
1,000	Lethal within minutes
Reference: ATSDR Toxicological profile of chlorine https://www.atsdr.cdc.gov/toxprofiles/tp172.pdf (accessed 06/2023), originally from Ellenhorn and Barceloux 1988.	

Routes of exposure

- As a gas at ambient temperatures, inhalation and eye exposure to chlorine are the most likely routes of exposure.
- Skin tends only to be affected if exposed to concentrated chlorine gas; if the skin is wet/moist or broken, or in the immediate vicinity of a release of pressurised liquid chlorine.
- Significant ingestion is unlikely because chlorine is a gas at room temperature.

Table 2. Signs and symptoms of chlorine exposure

Route	Signs and symptoms – see annex for detailed signs and symptoms
Inhalation	Irritation of eyes and nose with sore throat, cough, chest tightness, headache, fever, wheeze, tachycardia and confusion. Chemical pneumonitis, tachypnoea, dyspnoea and stridor due to laryngeal oedema may follow. Pulmonary oedema with increasing breathlessness, wheeze, hypoxia and cyanosis may take up to 36 hours to develop. Severe injury may result in long-term respiratory tract damage including persistent hoarseness, pulmonary fibrosis and chronic obstructive pulmonary disease. Secondary bacterial infection of the respiratory tract may occur.
Ocular	Eye exposure may cause irritation and burning sensation, pain, lacrimation, conjunctivitis and blepharospasm. Corneal injury, which is usually superficial, may occur.
Dermal	Skin exposure to gaseous chlorine may cause erythema, pain and irritation. Cutaneous burns may occur at high concentrations or following exposure to liquid chlorine.
Ingestion	Swallowing chlorine contaminated saliva can irritate the digestive tract, causing nausea, abdominal discomfort and vomiting.
	Source: UK chemical compendium, UKHSA. https://assets.publishing.service.gov.uk/media/5d53e4d0ed915d763e36a989/Chlorine_incident_management_PHE_2019__2_.pdf

C.4 Triage & assessment

Initial Actions:

- Remove patients from the affected area of exposure.
- Prioritise airway management and ensure patients have adequate ventilation.

4.1 Risks for first responders at the scene

A risk assessment should be carried out and appropriate controls and personal protective equipment (PPE) should be implemented (the risk can change depending on the amount of chlorine released, the environment of the release and the likelihood of ongoing release), including airway and ocular protection.

4.2 Decontamination

Decontamination may not be required; however, gaseous chlorine will irritate the skin at high concentrations and liquid chlorine may cause cutaneous burns. This should be considered when deciding on the need for disrobe and decontamination. Where appropriate, decontaminate as rapidly as possible, including self-decontamination where possible and supported where needed. (13)

Step 1 - Remove clothing and double-bag it, then seal and store it safely.

Step 2 – Commence wet decontamination, using copious amounts of water and a material such as a cloth or sponge.

Step 3 – Continue irrigation preferably for a minimum of 10-15 minutes. Pay special attention to skin folds, fingernails and ears.

Eye decontamination

Remove contact lenses if present and immediately irrigate the affected eye thoroughly with 1,000 mL 0.9% saline (e.g. by an infusion bag with a giving set), preferably for a minimum of 10–15 minutes. If saline or other appropriate solutions are not available, tap water may be used.

4.3 Triage for ongoing care

Individuals who have either ongoing or resolved mild irritation to mucous membranes (eyes, nose, throat) can usually be discharged/released. Patient records should be retained and those exposed should be advised to contact the emergency room or hospital if symptoms increase or do not resolve within a day.

Symptomatic patients, particularly those with respiratory features, should be medically assessed in hospital. All children (especially < 1 year) should be fully medically assessed in hospital. In symptomatic patients prior to transit:

Maintain a clear airway and ensure adequate ventilation. Check vital signs and cardiac rhythm. Administer oxygen to achieve adequate oxygenation. If patients have features of bronchospasm or obstructive symptoms, nebulised bronchodilators and steroids may be considered.

C.5 Clinical management in hospital

Clinical assessment is required to identify the scale of injury and the appropriate management steps to be taken on an individual basis.

Hospital care

- Monitor vital signs and cardiac rhythm.
- Oxygen should be given to anyone with pronounced irritation symptoms, dyspnoea, or hypoxia. Humidified oxygen therapy is preferred.
- Observe for at least 4 hours after exposure. Asymptomatic patients can then be considered for discharge with advice to return if symptoms develop.
- Symptomatic patients, particularly those with respiratory features, should receive a chest X-ray, full blood count, liver function tests, urea and electrolytes, calcium and blood gases. If no moderate or serious symptoms are presented and chest X-ray and blood gases are normal after 6–12 hours, they can be discharged with instructions to return if symptoms occur/worsen and to avoid exercise or strenuous activity for at least 24 hours.
- Individuals who have or have had moderate or severe symptoms (severe cough, haemoptysis, dyspnoea, bronchospasm, laryngospasm, decreased oxygen saturation, reduced general condition, pulmonary oedema): Observe/monitor in hospital for at least 24 hours.
- Perform a 12-lead ECG in all patients with ongoing respiratory symptoms.
- Check cardiac rhythm, QT interval and QRS duration.
- Consider repeating the ECG in ANY OF the following circumstances:
    - the initial ECG is abnormal,
    - the patient is symptomatic,
    - the recommended observation period (see above) is not yet complete.
- Bronchodilators for obstructive symptoms may be used in standard doses with inhaled glucocorticoids where required.
- Rest with sedation if needed in a comfortable, semi-sitting position.
- Consider early intubation in patients with signs of upper respiratory tract injury/obstruction, respiratory failure, or hypoventilation. Obtain early anaesthesia/intensive care review and advice. In severely affected patients, especially those with tachypnoea, stridor or upper

airway damage, critical care input is essential with urgent assessment of the airway. A supraglottic-epiglottic burn with erythema and oedema is a sign that further oedema will occur that may lead to airway obstruction. It is an indication for consideration of early endotracheal intubation or tracheostomy.

- Children are at increased risk of airway obstruction and treating clinicians should have a low threshold for establishing a protected airway.
- Treat pulmonary oedema and/or acute lung injury conventionally. In case of pulmonary oedema: High pressure ventilation with a continuous positive airway pressure (CPAP) mask or high positive end expiratory pressure (PEEP) ventilator. Use a low tidal volume (6–9 ml/kg). Use of diuretics is contraindicated as it may lead to hypovolemic and hypotensive effects.
- Fluid therapy: Give with caution due to the risk of fluid accumulation in the lungs due to increased capillary permeability.
- Monitor for secondary infection and ARDS and treat appropriately. Antibiotics will be required if pneumonia develops.
- ECMO (Extracorporeal membrane oxygenation) may be needed in single cases of respiratory failure as a last resort, for example pending lung transplantation.

Patients should be advised on discharge to seek medical attention if symptoms subsequently develop, for example developing a cough and/or shortness of breath during the first 24 hours can be signs of pulmonary oedema, which warrants further medical attention.

C.6 Key references

ATSDR: https://wwwn.cdc.gov/TSP/MMG/MMGDetails.aspx?mmgid=198&toxid=36

Milanez S. Chlorine. In Gupta RC (ed.) Handbook of Toxicology of chemical warfare agents. 3rd edition. Academic Press, 2020.

Zellner T, Eyer F. Choking agents and chlorine gas – History, pathophysiology, clinical effects and treatment. Toxicology Letters 2022; 320:73-79.

Achanta S, Jordt SE. Toxic effects of chlorine gas and potential treatments: a literature review. Toxicol Mech Methods 2021;31(4): 244-256.

Nambiema, A., et al. (2022). "Human chlorine gas exposition and its management-an umbrella review on human data." Critical Reviews in Toxicology 52(1): 32-50.

Russell, D., et al. (2006). "Clinical management of casualties exposed to lung damaging agents: A critical review." Emergency Medicine Journal 23(6): 421-424.

Huynh Tuong, A., et al. (2019). "Emergency management of chlorine gas exposure-a systematic review." Clinical Toxicology 57(2): 77-98.

Martinez, T. T. and C. Long (1995). "Explosion risk from swimming pool chlorinators and review of chlorine toxicity." Journal of Toxicology - Clinical Toxicology 33(4): 349-354.

White, C.W. and Martin, J.G. (2010). Chlorine gas inhalation: human clinical evidence of toxicity and experience in animal models. Proceedings of the American Thoracic Society . 2010 Jul;7(4):257-63. doi: 10.1513/pats.201001-008SM.

Public Health England. Chlorine Incident Management. PHE publications gateway number: 2014790. Published: August 2019.

Irritant gasses – treatment recommendation in case of poisoning. From the Norwegian Poison Information Centre. Revised 2022.

Major incidents involving exposure to chlorine. From the Norwegian Poison Information Centre. Published June 28th 2022

UK compendium of chemical hazards – Chlorine: health effects, incident management and toxicology. https://www.gov.uk/government/publications/chlorine-properties-incdent-management-and-toxicology

Chemical Agents – Nerve agents

D.1 Introduction

This part of the document summarises the potential health impacts and clinical management for people exposed to nerve agents. It should be used alongside any domestic advice and guidelines.

The nerve agents are produced exclusively for use as chemical warfare agents. They are organophosphates and therefore have the same general mechanism of action as pesticides (acetyl cholinesterase inhibitors) but are more toxic. Important properties such as volatility, persistence (a persistent agent remains in the contaminated area for a long time) and time of aging (see section D.2 "Aging") vary between the different agents.

Examples of nerve agents (NATO terms in parentheses):

Nerve agent	**CAS number**
Tabun (GA)	77-81-6
Sarin (GB)	107-44-8
Binary sarin (GB2)	
Soman (GD)	96-64-0
Ethyl sarin (GE)	1189-87-3
Cyclosarin (GF)	329-99-7
VX	50782-69-9
Binary VX (VX2)	
VG	78-53-5
Novichok agents	

D.2 Properties of the agents

Table 1. describes the different physicochemical properties of the nerve agents which are crucial for the method of dispersion, how efficiently they are dispersed and their persistence in the environment (particularly V agents and Novichoks, which can persist for days or months, depending on the environmental conditions). The nerve agents are present in liquid form at normal temperatures. They may be odourless or have a fruit-like odour, depending on the type of agent and impurities in the product. The physicochemical properties can be changed by additives.

Table 1 Physicochemical properties for some common nerve agents (1,2)

Agent	Odour	Boiling point (°C)	Volatility (g/m3 at 25°C)	Persi-stent *	LC_{t50} (mg min/m3)	LD_{50} Skin (mg/kg)	LD_{50} Oral (mg/kg)	Time for aging**
G								
Tabun (GA)	Fruity	240	Medium	No	135-400	14-57	0,6-5	> 14 hours
Sarin (GB)	None	147	High	No	35-100	14-28	0,14	5 hours
Soman (GD)	Fruit, cam-phor	198	Medium	No	20-100	1-18	0,14	2-4 min
V								
VX	None	298	Low	Yes	10-50	0,03-0,3	0,07	24-48 hours

Physicochemical properties (e.g. persistence) can be changed by additives. ** See section 2.1 for a description of aging. Estimated human *in vivo* values.

Nerve agents are generally divided into two groups:

- G-agents are relatively volatile, less persistent and emit a very hazardous vapour which poses a risk via inhalation
- V-agents are far less volatile, persistent and vapours normally pose a lower risk but are primarily a contact hazard

There are also the Novichok compounds (A-agents) which are newer and less characterised than other nerve agents. For further detail, see Annex 4.

Mechanism of action

The enzyme acetylcholinesterase (AchE) hydrolyses the transmitter substance acetylcholine (Ach) in the neuro-synaptic cleft. The nerve agents inhibit AchE and cause excess Ach to accumulate. This causes persistent stimulation of cholinergic receptors. Ach is a neurotransmitter both in the central nervous system (CNS) and in the peripheral nervous system (PNS). The effects are transferred via muscarinic and nicotinic receptors. Nerve agents are lipophilic and can cross the blood-brain barrier, causing neuro-irritability.

Aging

The nerve agents bind to AchE so that the enzyme is inactivated. The bond is initially reversible with the addition of oximes (e.g. pralidoxime), which remove the organophosphate nerve agent from AchE resulting in the enzyme slowly reactivating. However, over time the organophosphate nerve agent-AchE bond becomes more stable, a process called aging. This leads to irreversible binding between nerve agents and enzyme, and AchE can no longer be reactivated. To regain activity, a new enzyme must be synthesized in the liver. The variation in the aging rate of the various nerve agents is considerable (see Table 1). For soman, the aging happens quickly.

D.3 Health impact of nerve agent exposure

Routes of exposure

Table 2. Routes of exposure for nerve agents

Route of exposure	Effects
Inhalation	Nerve agents inhaled via aerosol spray/vapour are rapidly absorbed through the lungs.
Dermal/ocular absorption	Rapid absorption of nerve agents through skin or eyes can occur. However, skin absorption can be delayed for some nerve agents. Both vapor and liquid forms of nerve agent can penetrate rapidly through clothing and skin.
Ingestion	Ingestion is typically through contaminated food or drink. Oral absorption is significantly lower and slower than inhalation. Symptoms will occur later than with inhalation, but depending on the dose, symptoms may still be severe.

People exposed to nerve agents must have contaminated clothing removed as soon as possible and then be decontaminated (flushed with water, possibly also using soap – see sections 4.2 and 6.2 for more information) before transport to hospital, as clothing provides an additional source of exposure and can cause secondary contamination of healthcare staff. In patients who have only been exposed to nerve agent vapor, removal of clothing ("minimum decontamination") is usually sufficient before transport to hospital as soon as possible.

General signs and symptoms

The symptoms of exposure are listed below and may be mild, moderate or severe, with effects presenting within seconds/minutes/hours depending on the agent, its concentration and any underlying health issues of the individual.

Symptoms may include some or all of the following:

- Lacrimation, miosis (can occur rapidly following exposure), visual disturbances, salivation, eye pain, cough, mild bradycardia, sweating, fasciculation, abdominal pain, vomiting, nausea, bradycardia, rhinorrhoea.
- Dyspnoea, chest tightness, tremor, diarrhoea and incontinence, pronounced bronchial secretion, bronchial constriction, breathlessness, apnoea, pulmonary oedema, unresponsive pupils, ataxia, convulsions, coma, central and peripheral inhibition of respiration and circulation.
- Systemic absorption will rapidly produce systemic features. Death can occur within few minutes at a sufficiently high concentration.

Neuropathological damage in the Central Nervous System

Irreversible neuropathological damage to the central nervous system has been demonstrated. The injuries correspond to brain injuries that are observed after epileptic seizures. These substances, which cross the blood-brain barrier, can lead to neurotoxicity and fits/seizures which can contribute to long term neurological injury. Brain injuries have been detected in laboratory animals, including primates, who have had prolonged attacks (over approximately 45 minutes) of ataxia and muscle twitches ("epileptiform seizures") during exposure to nerve agents.

The mechanisms of neuropathological damage have not been fully characterized but appear to be related to the epileptiform seizures that exposure to nerve agents may cause. It is therefore extremely important to treat any epileptiform seizures with drugs that reduce brain activity (see below).

D.4 Triage and assessment

This section aims to separate the people exposed to nerve agents who can be decontaminated and triaged from those who need transporting to an acute hospital for treatment. At arrival on the scene of a potential nerve agent exposure, the below steps should be followed.

Risk for first responders at the scene

- Emergency responders need to ascertain if the area is contaminated and carry out a risk assessment (the environmental conditions must also be considered, as these can affect the dispersion of the nerve agent, which are heavier than air and can accumulate along low-lying areas). Responders require ongoing support and decontamination.
- Responders should approach the affected area/people with caution whilst wearing PPE (including equipment, clothing covering exposed skin, airway and ocular protection and support for donning and doffing the protective clothing).
- In addition, responders should only remain in the area for a limited time, appropriate for the protective equipment being used (which may be temperature-dependent).

Unprotected personnel must not enter the contaminated area. The emergency response team on site establishes danger zones (hot, warm, cold zones) and only emergency personnel with adequate protection (fire and rescue) will evacuate patients to decontaminate and triage on the border between hot and cold zones. If they do enter the area before it is known to be contaminated, they should be considered potentially contaminated and hence, as casualties.

Resuscitation

- All exposed persons should be transported to a non-contaminated safe area.
- Encourage those who are able, to remove themselves from the scene and remove clothing. "Walking contaminated" – the names of exposed people must be recorded in case they become subsequently unwell and need to be decontaminated.
- Triage of exposed individuals.
- Depending on the severity of the case, first aid should not be administered after decontamination, but during or at the same time as decontamination.
- Resuscitate (airway, breathing) and exclude opioid poisoning by use of antidotes (e.g. naloxone).
- Administer atropine (see below for details). If available, auto-injectors can also be used at the scene. Auto-injectors containing 220 mg obidoxime and 2 mg atropine may be used instead of the initial dose of atropine and obidoxime. Administer to adult patients who require atropine ("wet lungs"/crackling sounds over the lungs and/or bradycardia).

- Perform dry decontamination as soon as possible (remove clothing and double bag it).

Decontamination

Decontaminate exposed people as soon as possible after they have been removed/left the contaminated area by disrobing, followed by the best form of decontamination (see below) that is readily available as timely decontamination is critically important.

Disrobe

The disrobe process is highly effective at reducing contamination with HAZMAT/CBRN material when performed as soon as possible after exposure. Therefore, disrobing must be considered the primary action following evacuation of exposed people from a contaminated area. Where possible, disrobing at the scene should be conducted by the casualty themselves and should be systematic to avoid transferring any contamination from clothing to the skin. Avoid pulling clothes over the head of the exposed person; clothes should be cut and removed instead. Consideration should be given to ensuring the welfare and dignity of casualties as far as possible.

Improvised decontamination

Improvised decontamination is an immediate method of decontamination prior to access of specialised resources. This should be performed on all contaminated casualties, unless medical advice is received to the contrary. Improvised dry decontamination should be considered for nerve agents as they are not corrosive. Improvised wet decontamination can be used as alternative. For further detail, see Annex 4.

D.5 Clinical Management

Hospital care

If post-exposure initial resuscitation and triage indicate the need for ongoing clinical treatment, hospital treatment should start by seeking to minimise further contamination of that person by individual decontamination as appropriate, while establishing ongoing treatment with atropine as an infusion within maximal daily dose limits. For further detail on decontamination, see Annex 4.

Treatment at hospital

1. Atropine

Give atropine immediately. Dosing: Adults 2 mg IM or slowly IV. Children 0.05 mg/kg. If no immediate effect, atropine is repeated in doubling doses every 5 minutes until full atropinisation ("dry lungs"). Further treatment with atropine will depend on the degree of poisoning, the type of nerve agents, etc. Therefore, the amount of atropine should be titrated according to symptoms. Further

dosing may be given as infusions: e.g. 0.3 x total initial dose per hour. Evaluate effect on an ongoing basis and titrate to clinical response. Give a new bolus and increase the infusion in case of increasing crepitations over the lungs. It is very important to administer an adequate amount of atropine. High doses may be required. Atropine can be administered subcutaneously if intravenous access is difficult. Atropine must be discontinued gradually. In severe poisonings, pneumonia often develops. It will then be difficult to dose atropine based on bronchial secretion. Heart rate can be an indicative parameter in such cases.

2. Obidoxime or pralidoxime (reactivator)

An oxime should be administered as soon as possible (e.g. obidoxime or pralidoxime) if available. Even if oxime treatment is not started immediately, it should be given. To benefit from the oxime treatment, it is important that adequate doses are administered – seek specialist advice for dosage by chemical agent as titration to clinical response may be required.

3. Benzodiazepines

In case of severe poisoning, benzodiazepines should be given. They can be co-administered with atropine in an autoinjector. Dosing example: Adults 10 mg IV diazepam. With the onset of convulsions, this is repeated until the convulsions cease. Children 0.05-0.3 mg/kg IV (age 30 days to 5 years: Maximal dose 5 mg. Age 5 years and over: Maximal dose 10 mg). With the onset of convulsions, repeated doses are given. Other benzodiazepines may also be used. Midazolam is a good alternative to diazepam. For refractory epilepsy-like seizures with competent staff available, thiopental (Pentothal sodium) or propofol may be used as in status epilepticus. Traditional antiepileptic drugs (valproate, carbamazepine or phenytoin) probably have little effect on nerve gas-induced epileptiform activity.

If the patient's eyes have been exposed to nerve agent in liquid form, rinse the eyes with water for at least 10-15 minutes. For further detail, see Annex 4.

Clinical tests

The activity of AchE in plasma or erythrocytes is an indicator of poisoning with organophosphates. It should be noted that this test is for confirmation, not diagnosis, and requires further tests to identify the agent, if available. Ideally, chemical identification of the chemical should be undertaken, noting that any equipment used such as mass spectrometers, will become contaminated and may not be subsequently reused.

D.6 Key references

Chemical weapon data. Database from the Norwegian Defence Research Establishment. PB 25, 2007 Basement.

Sidell F. Nerve agents. 5; Tuorinsky(Red): Medical aspects of chemical and biological warfare. Textbook of military medicine. 2007. NBorden institute. Walter Reed Army Medical Center. Washington DC

UK Chemical Compendium – Nerve agents: Incident management. https://assets.publishing.service.gov.uk/media/5a823314e5274a2e8ab5803e/Nerve_agents_incident_management_201117.pdf

Worek F, Reiter G, Eyer P, Szinicz L. Reactivation kinetics of acetylcholinesterase from different species inhibited by highly toxic organophosphates. Arch Toxicol 2002; 76(9): 523-9.

Marrs TC, Maynard RL, Sidell FR. Chemical warfare agents. Toxicology and treatment. 1996 JohnWiley and sons. Baffins lane, Chichester, Wesr Sussex PO19 1UD, England.

Bakshi KS, Pang SNJ, Snyder R. Review of the U.S. army's health risk assessments for oral exposure to six chemical warfare agents. J Tox Envir Health 2000; 59(5-6): 281-526.

WHO 2003. Public health response to biological and chemical weapons. Draft May 2003.

Chebabo SR, Santos MD, Albuquerque EX. The organophosphate sarin, at low concentrations, inhibits the evoked release of GABA in rat hippocampal slices. Nerotoxicology 1999; 20: 871-82.

The European Agency for Evaluation of Medicinal Products (EMEA). Pre-authorisation Evaluation of Medicines for Human Use. 2003. EMEA/CPMP Guidance Document on the Use of Medicinal Products for the Treatment of Patients Exposed to Terrorist Attacks with Chemical Agents. Accessed October 2003.

Nozaki H, Aikawa N, Fujishima S, Suzuki M, Shinozawa Y, Hori S, Nogawa S. A case of VX poisoning and the difference from sarin. Lancet 1995; 346: 698-9.

Rotenberg JS, Newmark J. Nerve agent attacks on children: diagnosis and management. Pediatrics 2003; 112(3): 648-58.

Lifshitz, Shahak E, Sofer S. Carbamate and organophosphate poisoning in young children. Pediatric Emerg Care 1999; 15: 102-3.

ATSDR (Agency for toxic substances and disease registry). Medical management guidelines (MMGs) for nerve agents Tabun (GA); sarin (GB), soman, (GD) and VX. https://www.atsdr.cdc.gov/mhmi/mmg166.pdf. Available 2003-12-22.

Briefing note for emergency departments – management of suspected Novichok poisonings. https://assets.publishing.service.gov.uk/media/5b8ff89fe5274a0bdab54b55/ED_briefing_note_nerve_agents.pdf

Ellenhorn MJ, Schonwald S, Ordand G, Wasserberger J. Ellenhorn's Medical Toxicology. Diagnosis and Treatment of Human Poisoning. 2nd Edition. Baltimore: Williams & Wilkins, 1997.

Nozaki H, Hori S, Shinozawa Y. Relationship between pupil-size and acetylcholine esterase activity in patients exposed to sarin vapor. Intensive care Med 1997; 23, 1005-7.

Aas P, Myhrer T, Heyerdahl F. Drugs in supplementary medical treatment for nerve gas poisoning - relevant drugs in the protection of the brain. FFI Report 2009/01858. http://www.ffi.no/no/Rapporter/09-01858.pdf

Aas P, Jacobsen D. Poisoning with nerve gas. (Nerve gas – guidelines for care of victims of terrorism) (review). Tidsskr Nor Laegeforen 2005; 125: 731-5.

Vale, A.J., Marrs, T.C and Maynard, R.L. (2018). Novichok: a murderous nerve agent attack in the UK. Clinical Toxicology. 2018 Nov;56(11):1093-1097. doi: 10.1080/15563650.2018.1469759.

Chemical Hazards Emergency Medical Management (CHEMM): 4th Generation Agents: medical management guidelines. 2019. https://chemm.hhs.gov/nerveagents/FGA.htm

Morimoto F, Shimazu T, Yoshioka T. Intoxication of VX in humans. The American Journal of Emergency Medicine. 1999. Sep;17(5):494-494. doi: 10.1016/S0735-6757(99)90258-9.

Annex 1. Orthopoxviruses

Agents and transmission mode

Smallpox & Vaccinia

Smallpox spread from person to person is principally respiratory; contact with infectious skin lesions or scabs is an uncommon mode of transmission.

Vaccinia virus is the live-virus component of smallpox vaccines. Rarely, infection can occur from skin contact with fluid or crust material from the inoculation lesion of someone recently vaccinated against smallpox. Human contact with animals infected with vaccinia-like viruses has resulted in zoonotic infections in Colombia, Brazil, and India.

Mpox

After zoonotic transmission, mpox spreads from person to person via infectious respiratory secretions or through direct contact with infectious skin lesions (including scabs). African rodents and primates may harbour the virus and infect humans, but the reservoir host is unknown.

Cowpox

Cowpox infection occurs after contact with infected animals; person-to-person transmission has not been observed.

Pathogenesis

Poxviruses are the largest mammalian DNA viruses with the developmental cycle taking place in the cellular cytoplasm. These viruses produce a wide array of proteins that facilitate the synthesis of viral mRNAs outside the nucleus, replicate viral DNA, and assemble complex virions. Additionally, they play a role in regulating various interactions between the virus, individual cells, and the infected host organism. The genus *Orthopoxvirus*, within the family *Poxviridae,* is the most extensively studied due to its inclusion of four human-pathogenic species: variola virus (VARV), which causes smallpox; monkeypox virus (MPXV); cowpox virus (CPXV); and vaccinia virus (VACV). These orthopoxviruses are immunologically cross-reactive and cross-protective, so that infection with any member of this genus provides protection against an infection with any other member.

Orthopoxviruses penetrate the respiratory tract and rapidly pass into local lymph nodes. After a short period of viremia, the infection enters in a latent asymptomatic period which usually lasts from 4 to 14 days, during which the virus multiplies in the reticuloendothelial system. Thereafter, another brief period of viremia precedes the prodromal phase, during which the virus multiplies in the mouth and pharynx mucous membranes. The virus invades the capillary epithelium of the derma, leading to the development of typical lesions. Oropharyngeal and skin lesions contain abundant viral particles, particularly during the early illness phases. In fact, during this early stage, the virus may

also be detected in urine and conjunctival secretions. The lymph nodes, liver, spleen, bone marrow, kidneys, and other viscera may also contain large virus amount.

The initial migration of infected macrophages to lymph nodes triggers the production of cytotoxic T and B cells, in order to contain the spread of infection. Subsequently, neutralizing antibodies are typically secreted during the first week of illness but may be delayed in severe infections. By day 16 of the infection, haemagglutination-inhibition antibodies are detectable, and complement-fixation antibodies by day 18. Neutralizing antibodies may be detectable for many years, whereas levels of haemagglutination-inhibition and complement-fixation antibodies begin to decrease after one year. The correlation between humoral antibodies and protection from smallpox is not completely understood [1].

Reservoir

Orthopoxvirus species are named primarily according to the hosts from which they were first isolated and identified. However, the name does not necessarily represent its natural reservoir or complete host range. Despite a large number of studies, little is known about the primary hosts and reservoirs of zoonotic orthopoxviruses in nature, or their transmission and maintenance cycles. In terms of host range, orthopoxviruses can either be highly specialized and restricted to specific hosts, or act as generalists with a broad host range. For example, VARV is a highly specialized virus that exclusively infects humans. In contrast, MPXV, CPXV, and VACV are generalist zoonotic orthopoxviruses capable of infecting multiple mammalian species and occasionally spilling over into humans. The evolution of generalist pathogens requires the successful crossing of host transmission barriers. These barriers include geographical, ecological, and behavioural constraints that isolate a virus from potential host species. Additionally, virus-host cell incompatibilities, such as tissue tropism, receptor binding differences, genome replication, production, and shedding of infectious particles, play a role. Mechanisms of host immune evasion also contribute, involving cellular defences or responses that limit infection and/or allow the virus to evade the host's innate immune system. To overcome these barriers, orthopoxviruses have different biological features that can synergistically contribute to the transmission to, and exploitation of, a broad range of new hosts species as observed for CXPV, MPXV, and VACV.

Incubation and communicable periods

Table. Incubation and communicable period of Orthopoxviruses infections

Orthopoxvirus	Smallpox	Mpox	Cowpox, vaccinia and similar orthopoxviruses
Incubation period (days)	7-19	5-17	2-4

Transmission period	Smallpox patients became contagious once the first sores appear in their mouth and throat (early rash stage). They transmit the virus by coughing or sneezing, releasing droplets from their nose or mouth that can infect others. They remain contagious until their last scab has fallen off.	From the time symptoms start until the rash has fully healed and a fresh layer of skin has formed.	There is no known evidence of human-to-human transmission of the cowpox virus (https://www.ecdc.europa.eu/en/cowpox). Vaccinia virus can be transmitted from a vaccine recipient to other persons through direct (skin-to-skin) contact via material from the unhealed vaccination site or through indirect contact by means of fomites. Until the vaccination scab falls off, a person who has been vaccinated can transmit vaccinia virus to others. (https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5619a4.htm)

- https://www.cdc.gov/poxvirus/diseases.html Access 21st May 2024
- Gigante CM, Gao J, Tang S, McCollum AM, Wilkins K, Reynolds MG, Davidson W, McLaughlin J, Olson VA, Li Y. Genome of Alaskapox Virus, A Novel Orthopoxvirus Isolated from Alaska. Viruses. 2019 Aug 1;11(8):708. doi: 10.3390/v11080708. PMID: 31375015; PMCID: PMC6723315.
- Breman JG, Henderson DA. Diagnosis and management of smallpox. N Engl J Med. 2002 Apr 25;346(17):1300-8. doi: 10.1056/NEJMra020025. Epub 2002 Mar 28.
- https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/smallpox-and-other-orthopoxvirus-associated-infections
- Breman JG, Henderson DA. Diagnosis and management of smallpox. N Engl J Med. 2002 Apr 25;346(17):1300-8. doi: 10.1056/NEJMra020025. Epub 2002 Mar

- https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/small-pox-and-other-orthopoxvirus-associated-infections accessed on 28th march 2022
- https://www.cdc.gov/smallpox/transmission/index.html accessed on 28th march 2022

Annex 2. *Yersinia pestis*

Name and nature of infecting organism

Plague is a bacterial disease that has played an important role in the history of Europe. Three different plague pandemics have occurred in the past centuries, the latest one at the turn of the 19th century, and all with significant mortality worldwide. Plague has been absent from Europe for over half a century now, but is still widespread in the Americas, Africa and Asia. On an annual basis, a few thousand cases are reported worldwide.

Efforts to use *Y. pestis* as a biological weapon date back centuries. In 1346 CE, the Tartar army conquered Caffa (now Theodosia, Ukraine) by launching plague-infected corpses over the city walls, causing an outbreak. During World War II, Japan's Unit 731 dropped plague-infected fleas over China, resulting in thousands of persons contracting plague. In the 1970s and 1980s, the Soviet Union produced large amounts of antibiotic-resistant *Y. pestis* intended for airborne dissemination as a bioweapon.

Natural transmission

Reservoir

The natural reservoirs for plague are rodents, which normally undergo a subclinical course of infection. Different species have different host competence, and the reservoirs may differ between foci. Other rodent species, and other mammals, may act as multiplication hosts that develop serious disease and die of plague, increasing the speed of transmission. Black rats (*Rattus rattus*) are notorious as an intermediary between natural reservoir hosts and humans.

Seasonal patterns can be observed but differ between regions. Natural hosts may remain infectious for a long time, but peridomestic rodents usually die quickly and are infectious during the period they are ill.

Transmission mode

Transmission between rodents occurs through bites from infected fleas, and this is also the most common way in which humans are infected, leading to bubonic plague. Different flea species have a different vector capacity. Human-to-human transmission is rare in the case of bubonic plague, unless (human) flea densities are very high. Plague may also be acquired by ingestion of infectious (rodent) meat, by predators or humans.

Primary pneumonic plague follows inhalation of infectious droplets, produced by other patients or accidental hosts that developed pneumonic plague (e.g. cats that hunted infected rodents). Humans with pneumonic plague are most infectious during the last hours before death, and fast spreading epidemics with human-to-human transmission may occur.

Risk groups

Persons are more at risk when they come into close contact with wild rodents or their fleas in natural plague foci. Plague should be considered in symptomatic travellers returning from areas where plague occurs.

Annex 3. Chlorine agents

Detailed Symptoms and Signs of Chlorine exposure

Inhalation

- Irritation of the nose, mouth and throat, cough, hoarseness, increased salivation, chest discomfort/chest pain, respiratory distress, dyspnoea, tachypnoea, haemoptysis, bronchoconstriction/spasm.
- Significant exposure can lead to rapid onset of severe respiratory distress with oedema of the larynx, laryngospasm with respiratory obstruction, cyanosis, hypoxia, toxic pulmonary oedema, ALI (Acute Lung Injury), ARDS (Acute Respiratory Distress Syndrome). The lower respiratory tract symptoms may develop more gradually, but with pulmonary oedema developing within 24 to 36 hours.
- Secondary bacterial infection of the respiratory tract may occur.

Ocular exposure

- Eye irritation with burning sensation, pain, lacrimation, conjunctivitis and blepharospasm.
- Corneal damage/wounds are usually superficial.

Dermal exposure

- High gas concentrations can affect exposed skin, with severe burning sensation, redness and possibly ulceration, especially if the skin is moist.

Other situations

- If chlorine is in its liquid state, condensed liquid or outflowing from an aerosol corrosive damage or cold damage may occur.
- Swallowing chlorine contaminated saliva can irritate the digestive tract, causing nausea, abdominal discomfort, vomiting.

Long-term effects

- There is a risk of chronic lung changes with impaired lung function (such as Reactive Airways Dysfunction Syndrome (RADS)) after an acute exposure. However, this is mainly seen in people who initially had moderate to severe respiratory symptoms.

Treatment of eye and skin exposure

Eye exposure

- Anaesthetise the eye with a topical local anaesthetic (e.g. oxybuprocaine, amethocaine or similar); however, do not delay irrigation if local anaesthetic is not immediately available. (UK chemical compendium, chlorine incident management)
- Make sure adequate irrigation has been performed, if not irrigate as needed with 1,000 mL 0.9% saline (e.g. by an infusion bag with a giving set) for a minimum of 10-15 minutes irrespective of initial conjunctival pH. Amphoteric solutions are available and may be used. A Morgan Lens may be used if anaesthetic has been given. Aim for a final conjunctival pH of 7.5–8.0. The conjunctivae may be tested with indicator paper. Retest 20 minutes after irrigation and use further irrigation if necessary (UK chemical compendium, chlorine incident management).
- Repeated instillation of local anaesthetics may reduce discomfort; note that prolonged use of concentrated local anaesthetics is damaging to the cornea.
- Check for corneal damage in patients with prolonged signs or symptoms. Consult an ophthalmologist if corneal damage is found.

Skin exposure

- Do NOT apply neutralising chemicals as heat produced during neutralisation reactions may cause thermal burns and increase any injury.
- Burns totalling more than 15% of body surface area in adults (more than 10% in children) will require standard fluid resuscitation as for thermal burns.
- Moderate/severe chemical burns should be reviewed by a burns specialist. Excision or skin grafting may be required.
- Other measures as indicated by the patient's clinical condition.

Annex 4. Nerve agents

General Information

V agents

Due to the lower volatility of V-agents compared with the other nerve agents, dermal exposure is the main route of exposure. Inhalation and eye exposure will only occur at higher temperatures or in an enclosed space. Ingestion of food or water contaminated with VX may occur. A very small exposure may be sufficient to cause severe poisoning several hours later. VX has been used as an assassination agent via skin contact (Vale et al, 2018) and via injection (Morimoto et al, 1999).

G agents

G agents (Tabun, Soman, Sarin and Cyclosarin) are volatile and therefore a major inhalation hazard; ocular exposure is likely and dermal absorption can also occur. The onset of features occurs very rapidly after inhalation (within seconds or minutes) and more slowly after skin exposure. Death can occur within minutes following exposure.

Novichok (A) agents

Novichok ("newcomer" also known as 4th generation nerve agents) is a newer class of nerve agents which are less well characterised than other known nerve agents. There are numerous different compounds within the Novichok category. Novichok agents are low volatility agents and most likely to be encountered in liquid form, but they can be converted into a powder-like solid form Exposure by any route can be highly toxic and very small amounts may be sufficient to cause severe poisoning. There is very limited published data on these agents, but they are thought to act similarly to other nerve agents. (US 4th Generation nerve agents: medical management)

Key features distinguishing Novichok agents from other nerve agents:

- In general, the latent period between dermal exposure and symptom onset may be longer for Novichoks than for VX and can be up to several days. Inhalational, ingestion, or large dermal exposures will have shorter latent periods.
- Novichoks are persistent and can remain on enclosed environmental surfaces for days or even many months.
- Bronchoconstriction has been a prominent feature of Novichok toxicity in animal studies but has not been observed in the very limited number of reported human cases.
- Seizure activity has been a prominent feature of Novichok toxicity in animal studies.

Novichoks may cause severe metabolic acidosis with markedly elevated serum lactate. People poisoned by Novichoks may require an extended duration of clinical support. Multiple casualties may strain local resources at the point of care. (US 4th Generation nerve agents: medical management)

Decontamination

Improvised dry decontamination

- Any available dry absorbent material can be used, such as kitchen towel, paper tissues (e.g. blue roll) and clean cloth.
- Exposed skin surfaces should be blotted/patted (i.e. use the absorbent material to press against the skin to absorb any liquid, do not smear or wipe), starting with the face, head and neck and moving down and away from the body (Compendium of Chemical Hazards: Nerve Agents Page 13 of 16 Incident Management).
- Blotting should not be too aggressive, or it could drive contamination further into the skin.
- All waste material arising from decontamination should be left in situ, and ideally bagged, for disposal at a later stage.
- Water should only be used for decontamination where casualty signs and symptoms are consistent with exposure to caustic or corrosive substances such as acids or alkalis. The use of water to decontaminate requires a clear, safe means of disposal for any contaminated run-off, as this could cause further contamination of people or the environment. In addition, the risk from hypothermia must be considered when disrobe and any form of wet decontamination is carried out.

Improvised wet decontamination

- Wet decontamination may be performed using any available source of water such as taps, showers, fixed installation hose-reels and sprinklers.
- When using water, it is important to try and limit the duration of decontamination to between 45 and 90 seconds and, ideally, to use a washing aid such as a cloth or sponge.
- Lukewarm water is preferred. Hot water will increase dermal uptake, while cold water can cause hypothermia.
- Improvised decontamination should not involve overly aggressive methods to remove contamination as this could drive the contamination further into the skin.
- Where appropriate, seek professional advice on how to dispose of contaminated water and prevent run-off going into the water system.

Additional notes:

- Following improvised decontamination, remain cautious and observe for signs and symptoms in the decontaminated person and in unprotected staff.
- If water is used to decontaminate casualties it will become contaminated and therefore hazardous, which could become a potential source of further contamination spread.
- All materials (paper tissues etc.) used in this process may also be contaminated and should not be used on new casualties, but double-bagged and disposed of via an appropriate route later.
- Consideration should be given to ensuring the welfare and dignity of casualties as far as possible. Immediately after decontamination the opportunity should be provided to dry and dress in clean robes/clothes.
- Clinical carers should wear personal protective equipment (PPE) and secondary carers must wear appropriate PPE for chemical exposure to avoid contaminating themselves. The area should be well ventilated.
- Patients with clinically significant hypoxia, bradycardia, and or hypotension require oxygen and atropine, with airway stabilisation as necessary, before decontamination.
- Decontamination is very important after exposure to VX or VG, as it should reduce the risk of late severe poisoning.

Treatment

Ocular exposure

- While ocular exposure is possible, it is highly unlikely to occur alone and will more likely be in combination with dermal/inhalational exposure.
- Remove contact lenses if present.
- Anaesthetise the eye with a topical local anaesthetic (e.g. oxybuprocaine, amethocaine or similar); however, do not delay irrigation if local anaesthetic is not immediately available.
- Immediately irrigate the affected eye thoroughly with 1,000 mL 0.9% saline or equivalent crystalloid (e.g. by an infusion bag with a giving set) for a minimum of 10-15 minutes. Amphoteric solutions are available and may be used. A Morgan lens may be used if anaesthetic has been given. If more appropriate solutions are not available, tap water may be used.
- Any particles lodged in the conjunctival recesses should be removed.
- Evaluate the eye further and consult an ophthalmologist depending on the clinical sign and symptoms.
- Other supportive measures as indicated by the patient's clinical condition.