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Review

The Role of the Clinical Laboratory in Managing Chemical or Biological Terrorism

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Background: **Domestic and international acts of terrorism using chemicals and pathogens as weapons have recently attracted much attention because of several hoaxes and real incidents. Clinical laboratories, especially those affiliated with major trauma centers, should be prepared to respond rapidly by providing diagnostic tests for the detection and identification of specific agents, so that specific therapy and victim management can be initiated in a timely manner. As first-line responders, clinical laboratory personnel should become familiar with the various chemical or biological agents and be active participants in their local defense programs.**

Approach: **We review the selected agents previously considered or used in chemical and biological warfare, outline their poisonous and pathogenic effects, describe techniques used in their identification, address some of the logistical and technical difficulties in maintaining such tests in clinical laboratories, and comment on some of the analytical issues, such as specimen handling and personal protective equipment.**

Content: **The chemical agents discussed include nerve, blistering, and pulmonary agents and cyanides. Biological agents, including anthrax and smallpox, are also discussed as examples for organisms with potential use in bioterrorism. Available therapies for each agent are outlined to assist clinical laboratory personnel in making intelligent decisions regarding implementation of diagnostic tests as a part of a comprehensive defense program.**

Summary: **As the civilian medical community prepares for biological and chemical terrorist attacks, improvement in the capabilities of clinical laboratories is essential in supporting counterterrorism programs designed to respond to such attacks. Accurate assessment of resources in clinical laboratories is important because it will provide local authorities with an alternative resource for immediate diagnostic analysis. It is, therefore, recommended that clinical laboratories identify their current resources and the extent of support they can provide, and inform the authorities of their state of readiness.**

Release of chemical and biological warfare agents threatens civilian populations throughout the world. Potential sources of exposure include accidental release from military factories and stockpiles, direct military attacks, industrial accidents, and intentional release as an act of terrorism *(1)*. Chemical warfare has been used by the military, such as the mustard gas attack by the Iraqi government on civilians in Iraq and Iran in 1986 *(2)*, and by terrorist organizations, such as the release of sarin by the Aum Shinrikyo cult in Matsumoto City (Japan) in 1994 *(3)*. Both of these incidents caused high morbidity and mortality. The use of biological agents as weapons has been limited. Major incidents have included the accidental release of anthrax from a military testing facility in the former Soviet Union in 1979 *(4)* and the intentional contamination of restaurant salad bars with *Salmonella* by a religious cult in Oregon in 1984 *(5)*.

Many of the attacks involving disease-producing organisms, or in some cases chemical agents, are first identified by local medical institutions. Rapid response to such attacks involves the timely administration of antidotes, especially for cyanides and nerve agents, or vaccines and antibiotics for smallpox and anthrax, respectively. Therefore, there might not be sufficient time for advanced analytical procedures to be performed in highly equipped government or military laboratories. The local laboratory will play an important role in providing rapid identification of the agent involved, which will influence the administration of an antidote or vaccine to the affected victims.

We will review selected agents that have previously been considered or used in chemical and biological war-

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fare, discuss the mechanisms by which these agents act, and outline the techniques used for their identification. We will also discuss current antidotal, antimicrobial, and vaccination treatment strategies and address specific analytical issues, such as specimen handling and the use of laboratory testing for the diagnosis and management of victims exposed to chemical or biological agents. Laboratory personnel should obtain funding from local authorities to defray the costs of specialized equipment, reagents, and personnel *(6)*.

Regardless of the type of agent used in an attack (i.e., chemical or biological), it is important to provide sufficient protection for laboratory personnel who will analyze suspicious environmental materials, air samples, and specimens collected from humans. Most hazardous-material-response teams are currently equipped with level A personnel protective equipment *(1)*. It is intuitive that local laboratory personnel who will be dealing with these attacks in support of the defense structure should be equipped with appropriate personnel protective equipment to prevent exposure to chemicals or organisms.

Chemical weapons are classified into four major categories based on their mode of action: nerve agents, blistering (vesicant) agents, pulmonary agents, and cyanides. We will review representative agents from each class of these chemicals (Table 1).

With regard to biological agents, the biological warfare programs of both the United States and the former Soviet Union had *Bacillus anthracis* (anthrax), botulinus toxin, and Venezuelan equine encephalomyelitis virus in their respective biological weapons arsenals. Additional agents used by the United States for biological weapons research programs before the 1970s were *Francisella tularensis*, staphylococcal enterotoxin B, *Brucella suis*, and *Coxiella burnetii*. The Soviet Union had focused on smallpox, plague, Q-fever, Marburg hemorrhagic fever, melioidosis, and typhus *(4)*. Table 2 lists the various bacteria and viruses that should be considered as possible agents in a bioterrorism attack. Because of their lethality and propensity for mass destruction, anthrax and smallpox will be highlighted in this review.

Chemical Agents

Measurement of various chemical warfare agents, and perhaps their degradation products, in victims can be useful for triaging patients, confirming exposure, and identifying the particular substances involved and the possible treatments available, including antidotal therapy. The role of the clinical laboratory is to provide rapid analysis of biological samples in situations for which timely results can affect patient treatment. Clinical laboratories should also collect, handle, and ship specimens in a manner appropriate for government and reference laboratories to conduct further analysis and confirmatory testing. Providing information on each chemical agent to the physicians treating potentially exposed individuals is also an important role for the clinical laboratory.

The use of poisons as weapons dates back to 431 BC, when sulfur was burned beneath the walls of the cities of Plataea and Belium in the Athenian and Spartan Wars *(7)*. Other notable incidents before the 20th century include the use of picric acid and sulfur by the British during the Boer and Crimean Wars. The most extensive use of chemical warfare occurred during World War I, when the

Table 1. Chemical warfare agents.

Name	Code	Chemical name	Site of action
Nerve agents			
Tabun	GA	Ethyl-*N*-dimethylphosphoroamidocyanidate	AchE
Sarin	GB	Isopropylmethylphosphonofluoridate	AchE
Soman	GD	Pinacolyl methylphosphonofluoridate	AchE
VX	VX	*O*-Ethyl *S*-[2-(diisopropylamino)ethyl] methylphosphonothiate	AchE
Vesicants			
Sulfur mustard	HD	2,2′-Dichlorodiethyl sulfide	Blistering agent; strong alkylating properties, affecting eyes, skin, and lungs
Nitrogen mustard	HN-1	2,2′-Dichloro-*N*-methyldiethylamine	Blistering agent; strong alkylating properties, causing systemic toxicity
Lewisite I	L1	Dichloro(2-chlorovinyl)arsine	Blistering agent, irritating to eyes and lungs
Pulmonary agents			
Phosgene	CG		Lung
Chlorine	CL		Lung
Cyanides			
HCN	AC	Hydrogen cyanide	Cytochrome oxidase
CK	CK	Cyanogen chloride	Cytochrome oxidase

Table 2. Biological warfare agents.[a]

Organism/Agent	Disease caused or common name	Biothreat[b] level
Variola major	Small pox	A
B. anthracis	Anthrax	A
Yersinia pestis	Plague	A
Clostridium botulinum toxin	Botulism	A
F. tularensis	Tularemia	A
Ebola filovirus	Ebola hemorrhagic fever	A
Marburg filovirus	Marburg hemorrhagic fever	A
Lassa arenavirus	Lassa fever	A
Junin arenavirus	Argentine hemorrhagic fever	A
C. burnetii	Q-Fever	B
Brucella species	Brucellosis	B
Burkholderia mallei	Glanders	B
Alphaviruses	Venezuelan encephalomyelitis or equine encephalomyelitis	B
Ricinus communis (castor beans)	Ricin toxin	B
Clostridium perfringens	Epsilon toxin	B
Nipah virus		C
Hantaviruses		C
Tick-borne viruses	Hemorrhagic fever and/or encephalitis	C

[a] Adapted from Khan et al. *(83)*.

[b] Category A agents pose the greatest threat because of their relative ease of transmission and high rate of mortality. Category B agents have moderate ease of transmission and morbidity with low rate of mortality. Category C agents refers to emerging pathogens and potential risks for the future.

Germans released chlorine gas against the Russians. Mustard gas was also introduced by the Germans during this period and was used by both armies, causing 1.2 million casualties *(7)*. The Germans introduced nerve agents in 1943 and used them to kill millions of people incarcerated in concentration camps. Although the use of chemical agents in warfare violated the Geneva Convention of 1925, the Italian army used mustard gas against Ethiopia in 1935, Japan conducted chemical attacks on the Chinese in 1937, and the Iraqi government carried out poison attacks on civilians in both Iraq and Iran in 1986 and on the Kurdish population in northern Iraq in 1988 *(2, 7, 8)*. The United States Congress ordered the destruction of the chemical weapon stockpile of the United States in 1986 *(9)*.

NERVE AGENTS

Nerve agents (Table 1) are organic esters of phosphoric acid derivatives (similar to the organophosphate pesticides) and elicit their toxic effects through inactivation of acetylcholinesterase (AchE)[3] *(10)*. The worldwide stockpile of nerve agents is estimated to be ~200 000 metric tons *(11)*.

[3] Nonstandard abbreviations: AchE, acetylcholinesterase; GC, gas chromatography; MS, mass spectrometry; MS-MS, tandem MS; and BT, biothreat.

Acute toxic manifestations of cholinergic crisis caused by nerve agents are similar to those caused by organophosphates, which include increased sweating and bronchial, salivary, ocular, and intestinal secretions; "pinpoint" pupils (miosis); intestinal hypermotility; bronchospasms; and bradycardia *(11–13)*. Muscular fasciculation, weakness, and paralysis also can be observed because of nicotinic effects on the synapse. Other health effects from exposure to organophosphates, including nerve agents and insecticides, are organophosphate-induced delayed neuropathy, subtle long-term effects on the nervous system (physiological and psychological), and intermediate syndrome (i.e., reversible muscular weakness) *(14)*.

The "G" agents [GA (tabun), GB (sarin), and GD (soman)] have a greater ability than VX to activate the nicotinic acetylcholine receptor ion channels *(11)*. The bonds formed between nerve agents and AchE can be rapidly hydrolyzed by oximes (RCH═NOH) such as obidoxime chloride or pralidoxime, which provides the rationale for the use of these drugs as antidotes *(13)*. Other approaches, such as immunotherapy (i.e., the administration of antibodies against poisons such as soman to reduce their toxicity), have been reported *(15)*. An indication of exposure to nerve agents and organophosphate pesticides is a rapid reduction in serum cholinesterase activity (pseudocholinesterase). Automated instruments, such as the Vitros (Ortho Clinical Diagnostics) and the aca® STAR™ (Dade-Behring), have assays available for measuring cholinesterase activity. Ratnaike et al. *(16)* also have described a method for measuring plasma cholinesterase with the COBAS-Bio centrifugal analyzer. Recovery of cholinesterase activity has been reported to range from 30 to 40 days for plasma and 90 to 100 days for erythrocytes *(17)*. Therefore, serum cholinesterase activity can be measured for assessment of acute exposure and erythrocyte enzymatic activity for evaluation of chronic exposure.

Meuling et al. *(18)* have reported an automated method for the determination of both acetyl- and pseudocholinesterase activity in hemolyzed whole blood. In this technique, butyrylthiocholine and acetyl-β-(methyl)thiocholine are used as substrates, each of which has >97% specificity for its intended enzyme. With regard to measuring the concentration of a particular nerve agent, gas chromatographs equipped with different detection modes (e.g., thermospray ionization detection, flame ionization detection, and nitrogen-phosphorous detection) can be used *(19)*. With gas chromatography (GC), the Kovats retention time index can be used to reliably screen samples for the presence of chemical warfare agents, including the nerve agents. The Kovats index relates the logarithm of the retention time of a substance, regardless of its chemical nature, to *n*-paraffins eluting before and after the peak of interest *(20)*. To increase the sensitivity of detection, various approaches, such as injection of large volumes of sample on the column, atomic emission detection, and GC coupled with ion mobility spectrometry, have

been taken *(19)*. The most preferred method of detection and confirmation of nerve agents is GC coupled with mass spectrometry (GC-MS). Both chemical and electron-impact ionization followed by tandem MS (MS-MS) have been used for identification of nerve agents *(21)*. Methods such as this have been touted as indispensable for the analysis of chemical warfare agents, but the complexity of the procedures and the time-consuming derivatization reactions are considered disadvantages *(19)*. Therefore, at present the conformation of a particular nerve agent must be determined in highly specialized laboratories, such as those managed by the military or other government agencies.

Nerve agents and the organophosphate pesticides have been measured using biosensor technology *(22)*, which includes a biological element (e.g., an enzyme or an antibody) and a detection element. The biological element of the biosensor can be AchE itself, which is commercially available in purified form. Various detection modes for enzymatic activity have been reported, including the use of electrodes to monitor changes in pH *(23)*, a light "addressable" potentiometric sensor *(24)*, ion-sensitive field-effect transistors *(25)*, and amperometric measurement of sequential enzymatic reactions catalyzed by AchE *(26)*. Recently, the degradation products of nerve agents have been successfully separated by reversed osmotic flow capillary electrophoresis *(27)*. This method was developed for environmental monitoring and is highly sensitive (detection limit, 75 μg/L in conductivity detection mode and 100 μg/L in ultraviolet detection mode) *(27)*. Anti-VX monoclonal antibodies have been used to measure low VX concentrations (~4 μg/L) *(28)*.

VESICANTS

Vesicants are also known as blistering agents because they irritate the skin, eyes, and lungs and may further affect other organs if absorbed into the body. This group of chemical warfare agents includes sulfur mustard, Lewisite [dichloro(2-chlorovinyl)arsine], and nitrogen mustards. Nitrogen mustards have not been produced in substantial quantities, and they have some medicinal use as anticonvulsant agents *(29)*. Toxic manifestations of sulfur mustard poisoning appear several hours after exposure *(9)*, whereas Lewisite readily irritates the skin on contact *(30)*. Sulfur mustard (Table 1) was first synthesized in 1854 and was used during World War I by the German army *(31)*. In the 1980s, sulfur mustard and, to a lesser extent, Lewisite were used by the Iraqi government against the Kurdish and Iranian populations and by the Soviet Union against the Afghani population. Mustard gas, named because of its distinctive odor, appears as "a poisonous cloud" in aerosol form. It is a strong alkylating agent with the ability to undergo nucleophilic substitution and hydrolytic reaction with other molecules, such as proteins *(32)*.

Sulfur mustard is considered a vesicant because of its blistering properties, which affect the skin, eyes, and respiratory tract *(29)*. Reporting on the treatment of 535 patients exposed to mustard gas in the Iraq-Iran War, Momeni et al. *(31)* observed erythema in 76%, bulla in 55%, and purpura in 1.1% of individuals. The authors considered lymphopenia the most serious clinical deficiency, which appeared in 7% of patients. Subepidermal bullae with mild dermal and epidermal necrosis were the primary pathological findings on the biopsy samples of the lesions *(31)*. Although it has generally been believed that there is no effective antidote for sulfur mustard, Sawyer et al. *(33)* recently reported that 1-thiocitrulline provides a high degree of protection against this agent. This protection evidently occurs through inhibition of nitric oxide synthase by arginine analogs such as 1-thiocitrulline.

It is generally accepted that Lewisite poisoning can be effectively treated by British Anti-Lewisite (2,3-dimercaptopropanol). This antidote also has been used against other poisons containing heavy metals (e.g., mercury, arsenic, lead, copper, and gold) *(30)*. The Allies first synthesized Lewisite during World War I. Despite the fact that it was not used in the war, it was considered the best arsenical war gas *(30)*. Lewisite is a racemic mixture of the *cis* and *trans* isomers, which are highly reactive because of the presence of various reactive groups (e.g., chlorine, trivalent arsenic, and multiple bonds). After contact with the human body, Lewisite is rapidly converted to the 2-chlorovinyl arsonous form, which produces severe irritation of the skin, eyes, and lungs *(30)*. Lewisite can be toxic to the liver and kidneys and may lead to permanent blindness if not decontaminated immediately *(34)*.

Because of the use of blistering agents during the Iran-Iraq War, various methods were developed to detect sulfur mustard in water and soil and in biological fluids collected from victims *(19)*. Both solid- and liquid-phase extraction procedures have been described for the isolation of sulfur mustard from environmental and biological specimens *(35, 36)*. The major hydrolysis product of sulfur mustard is thiodiglycol *(29, 37)*. Other metabolites of sulfur mustard that can be detected in the urine have been reported *(38)*. Analysis of sulfur mustard and its degradation products has been performed using capillary GC *(35)*, GC-MS *(36)*, and GC-MS-MS *(39)*. The GC-MS-MS method has been reported to be highly sensitive, with a detection limit of 0.1 μg/L compared with the GC-MS or capillary GC methods, which have detection limits of 2–5 and 45 μg/L, respectively *(35, 39)*. Hooijschuur et al. *(37)* recently reported the online coupling of reversed-phase microcolumn liquid chromatography and sulfur-selective flame photometric detection to measure sulfur mustard and thiodiglycol in aqueous samples. In addition to measuring sulfur mustard and its degradation products, adducts formed by alkylation of hemoglobin *(40)*, serum albumin *(41)*, and DNA *(42)* have been identified and considered as potential biomarkers for exposure to this vesicant. The hemoglobin adducts were measured by electrospray ionization liquid chromatography-MS-MS,

whereas analysis of the alkylated products of serum albumin involved the binding of ^{14}C-labeled sulfur mustard to the protein and MS analysis of the cysteine adducts *(40, 41)*. The detection of DNA-sulfur mustard adducts by ELISA using an antiserum produced in rabbits has been shown to have a detection limit comparable to that of an ELISA method that uses monoclonal antibodies *(42)*.

PULMONARY AGENTS

Pulmonary agents primarily affect the lungs and the ability to breathe. These agents are also referred to as choking agents and include chlorine gas, phosgene, diphosgene, and chloropicrin *(34)*. Of these, chlorine gas and phosgene exhibit their toxicity in the form of acute pulmonary edema accompanied by irritation of the nose, larynx, pharynx, trachea, and bronchi *(34)*. Chlorine is a greenish-yellow gas first used as a chemical warfare agent in World War I, and it is present in various industrial and household cleaning products. The interaction of chlorine gas with water produces hydrochloric acid (HCl) and hypochlorous acid (HOCl), which can damage tissues *(43)*. Because of its intermediate water solubility, chlorine affects both the upper and peripheral airways, causing immediate mucus membrane irritation, cough, hemoptysis, chest tightness, and shortness of breath *(43, 44)*. Toxic pulmonary edema is the main consequence of chlorine exposure, and treatment is mainly symptomatic with supplemental oxygen by mask, antitussives, and bronchodilators *(45, 46)*. In a worker who had been briefly exposed to chlorine gas in Australia, irritating cough was the main symptom observed. Interestingly, exposure to the chlorine gas had changed the color of the worker's underwear from blue to pink. This was attributed to the production of hydrochloric acid on the moist surface of his clothing, which was comparable to the surface of litmus pH paper indicating acid formation *(47)*.

Exposure to chlorine gas can be measured by automated portable sensors, which are based on electrochemical conversion of the gas molecule to two Cl^- ions. Alternatively, exposure to chlorine gas can be measured by its absorption in 1 mol/L sodium hydroxide, followed by the addition of hydrogen chloride and potassium iodide, and by the titration of chlorine with 0.01 mol/L sodium thiosulfate *(45)*. It has not been determined whether exposure to chlorine gas leads to increases of serum chloride. Currently, the main role of the clinical laboratory in cases of chlorine gas poisoning is to provide rapid blood gas analysis, which is continuously monitored during the supportive therapy of victims. Pulmonary function tests are important and are usually administered by other entities in the clinical setting. Pulmonary complications, mainly characterized by low residual volume, have been reported as long-term sequelae associated with chlorine gas poisoning *(48)*.

The other prominent choking agent is phosgene (carbonyl chloride), which was first synthesized in 1812. It can be produced by the combination of carbon monoxide, chlorine, and activated charcoal and appears as a colorless gas with the smell of fresh-cut grass *(49)*. Phosgene was used against the Allies in World War I and was responsible for >80% of the chemical agent-related deaths *(34)*. It currently has extensive use in various industries, e.g., in the production of dyes, plastics, polyurethanes, pesticides, and pharmaceuticals *(50)*. A choking sensation, cough, nausea, and headache are usually present <2 h after exposure to phosgene, which is considered the initial period of exposure *(34)*. In the latent period, which can be up to 48 h after exposure, the victim may be free of any symptoms of poisoning. Usually during the first 24 h after the latent period, severe pulmonary damage and edema occur, accompanied by hypoxemia and reduced plasma volume *(49)*. Some of the symptoms of phosgene poisoning appearing at this point include rapid respiration, painful cough, and cyanosis *(34)*. Death occurs as a result of hypoxemia, hypotension, and respiratory failure. One proposed mechanism for the toxicity of phosgene is its hydrolysis to HCl and CO_2, leading to local irritation and severe reflex vasoconstriction, both of which contribute to pulmonary edema. Death occurs as the result of subsequent events, mainly loss of plasma volume, hypoxia, shock, and cardiac failure *(49)*.

An alternative mechanism for phosgene-induced acute lung injury involves the direct changes made on the erythrocyte membranes by exposure to the gas *(50)*. Nevertheless, phosgene-induced neutrophil migration has been associated with extent of lung injury and mortality. Inhibitors of neutrophil influx, such as colchicine, may have potential antidotal value *(51)*. Another antidotal approach has been the use of hexamethylenetetramine, whose efficacy has been controversial *(52)*. However, prophylactic administration of this agent has been shown to provide substantial protection against the adverse effects of phosgene *(52, 53)*. Postexposure aminophylline therapy *(54)* and intratracheal administration of dibutyryl cAMP (which inhibits pulmonary endothelial or epithelial cell contraction and arachidonic acid production) have been shown to be protective against phosgene-induced pulmonary edema *(50)*. In the treatment of phosgene poisoning, Regan *(55)* has recommended early intubation and adequate blood volume monitoring followed by extended pulmonary function testing and chest x-rays (for up to 2 months). Currently, there is no method available for analyzing phosgene in biological fluids for diagnostic purposes. However, phosgene can be detected in air by portable gas detectors similar to the ones described for chlorine testing.

In preparing for a potential gas attack involving chlorine or phosgene, understanding the particular characteristics of each agent, such as odor and acute and delayed effects on the patient, may be of great value. The clinical laboratory should be prepared to analyze blood gases immediately on potential victims. It also has been reported that the protein concentration in bronchoalveolar

lavage is increased as a result of phosgene poisoning *(56)*. Therefore, the laboratory should validate bronchoalveolar lavage as an acceptable specimen for protein analysis and be prepared to provide this test as an indicator of the extent of edema.

CYANIDES

Cyanide and cyanogenic agents elicit their poisonous properties by affecting respiration and oxygen consumption, which leads to anoxia. The sources for cyanide poison are diverse, ranging from fruit pits, nuts, or seeds to industrial-based materials, such as those used in metal processing, electroplating, rubber and plastic production, insecticide and rodenticide production, chemical synthesis, and extraction of gold and silver ores *(57)*. Cyanide can also be produced at very low concentrations in vivo as a result of vitamin B_{12} metabolism or smoking. Cyanide can be ingested in many forms, including the halogenated form, salts (i.e., potassium, sodium, or calcium), or as hydrogen cyanide gas. Cyanide can also be generated in vivo as a result of the metabolism of cyanogenic plants or various pharmaceuticals such as sodium nitroprusside and laetrile *(57)*. Cyanide has been used in suicidal and homicidal deaths as well as in judicial executions. The Allies used cyanogen chloride (ClCN), a highly volatile liquid, and the gas form, HCN, in World War II *(29)*. In its gaseous form, cyanide is lethal within seconds after exposure, and it can cause death within minutes to several hours after ingestion of the salt form *(57)*. In a genetically determined fashion, 50% of the population is able to recognize the almond-like odor of cyanide at concentrations >1 ppm in air *(58)*.

Cyanide has a great affinity for iron in its ferric state (Fe^{3+}), thereby inhibiting mitochondrial cytochrome oxidase enzyme. Inhibition of this enzyme leads to failure of oxygen transportation and utilization by the cells, leading to death from cytotoxic anoxia *(59, 60)*. The main focus in the treatment of cyanide poisoning is to provide a large pool of oxidized iron to compete with the cytochrome oxidase for binding to the poison *(60)*. Nitrites can be used as antidotes to oxidize the hemoglobin in blood to methemoglobin, which can bind to cyanide to form cyanmethemoglobin. Various cobalt compounds such as cobalt EDTA and hydroxycobaltamine can also be used as antidotes; when hydroxycobaltamine is used, cyanide vitamin B_{12} (cyanocobalamin) is formed, which can be excreted by the kidneys *(60)*. Detoxification can also include administration of thiosulfate, which is incorporated into thiocyanate by rhodanese (a mitochondrial transsulfurase enzyme) and excreted in the urine. Although administration of oxygen alone in cyanide intoxication cases is not very useful, when combined with the above-mentioned antidotes, it can be highly effective *(60)*.

Although venous blood collected from cyanide-intoxicated patients has been said to appear pink or bright red because of reduced dissociation of oxygen, this may not always be true *(59)*. According to Troup and Ballantyne *(61)*, determination of cyanide concentrations in blood is useful for biochemical confirmation and after antidotal treatment and other therapeutic measures. Others have disagreed with this point based on the fact that the toxic burden is the amount of cyanide within the cells. Typical plasma and whole blood cyanide concentrations are 0.004 and 0.016 mg/L, respectively, in nonsmokers and 0.006 and 0.041 mg/L, respectively, in smokers. In cases of suspected cyanide poisoning, blood concentrations >2.5 mg/L are associated with coma and death *(51)*. Blood cyanide values measured in fatalities caused by ingestion are higher than in fatalities caused by inhalation of the drug *(58)*. Thiocyanate concentrations in plasma have also been measured in the plasma of poisoned patients; however, the use of these measurements in real-time management of patients is questionable. It is noteworthy that blood concentrations of cyanide either increase or decrease during storage, depending on the temperature *(62)*. Measurement of cyanide in various human tissues, especially in blood, has been described *(61)*. It is important to note that samples collected for cyanide analysis should be kept in sealed containers. This will assure that cyanide is not produced or lost and that results are not affected because of storage.

Various methods, such as the of the Conway microdiffusion dish or various aeration methods, have been described previously for extraction of cyanide from samples *(61)*. A head-space gas chromatographic procedure for the isolation and analysis of cyanide has also been described *(63)*. In this method, the detection limit was 0.005 mg/L, and the method was linear at concentrations of 0.005 mg/L to 1 g/L. Derivatization of cyanide to 1-cyano-2-benzoisoindole, using 2,3-naphthalenedialdehyde, followed by fluorometric detection (excitation wavelength, 418 nm; emission wavelength, 460 nm) was reported to be linear at concentrations of 0.002–1 mg/L *(64)*. Use of the Conway microdiffusion dish followed by ion-specific electrode *(65)* has also been reported for determination of blood cyanide concentrations. Determination of blood cyanide and thiocyanate by GC-MS after extractive derivatization has also been reported *(66)*. A simple, rapid, and inexpensive method for the detection of cyanide in blood is the Cyanotesmo test paper (available from Gallard-Schlesinger Industries) with a detection limit of 0.2 mg/L *(67)*.

Biological Agents

One major challenge confronting the clinical laboratory, especially the microbiology laboratory, is being prepared to respond to a bioterrorism event in a timely and efficient manner. The role to be played by the clinical microbiology laboratory is no different from its current role: to detect, recover, characterize, identify, and if possible, determine the susceptibility of the agent to antimicrobial agents. Complicating the role of the laboratory is the challenge that confronts laboratory personnel in suspecting and recognizing the current targeted agents.

Of the three major types of weapons of mass destruction, biological agents pose the greatest threat and are most likely to be used in the commission of a biocrime. Biological agents possess unique properties that enhance their attractiveness to individuals or groups wanting to inflict high morbidity and mortality on the human population (Table 3). For example, although anthrax is not spread from person to person, those individuals who are exposed to an aerosol containing the organism may develop illness within 72 h and up to 8 weeks after initial exposure.

The NATO Handbook on Biological Terrorism *(68)* lists 31 biological agents that have been targeted as most likely to be used in a bioterrorism attack. Of these, six have been designated as "high priority" agents: *B. anthracis*, the agent in anthrax; *Yersinia pestis*, the agent in plague; *F. tularensis*, the agent in tularemia; *Brucella* spp, the agents in brucellosis; botulinum toxin, produced by *C. botulinum*; and variola major (smallpox). These agents, with the exception of variola major, occur naturally in the environment and cause occasional episodes of infection in human and animal populations. Lower on the list of potential agents are glanders, typhus, Q fever, Venezuelan equine encephalomyelitis, and the hemorrhagic viruses such as the Marburg and Ebola viruses. Thus, any biological agent is capable of causing human disease and must be considered as a potential threat. Furthermore, anyone with a basic knowledge of microbiology, including an understanding of culture methods for the detection and recovery of these organisms, is potentially capable of producing and releasing an agent in an act of bioterrorism. Such an act occurred in 1984 when members of the Rajneeshi religious sect intentionally contaminated salad bars with *Salmonella typhimurium* in Dallas, Oregon with the intent of influencing local elections. Nearly 750 people became ill, but none died or required hospitalization *(69)*. Other such episodes could occur, but unlike the lethal consequences of smallpox and anthrax, agents such as *S. typhimurium* have a low virulence capacity, and outbreaks resulting from these strains are unlikely to cause panic or overwhelm the healthcare capacity of the medical community.

Table 3. Unique attributes of biological warfare agents.

- Pathogenic to humans, animals, and plants
- Environmentally stable because they are found naturally in the environment
- Effective at low doses
- Adaptable to weapon systems, including dispersal by aerosols
- Cause high rates of morbidity and mortality
- Difficult to diagnose and/or treat
- Relatively easy to obtain because of their natural occurrence
- Inexpensive compared with nuclear and chemical agents
- Adaptable to gene manipulation, which can lead to the creation of "designer" microbes with increased virulence and/or antimicrobial resistance
- Delayed symptoms, usually days to weeks after the attack

The frequency at which these organisms are encountered in the laboratory is extremely rare, and as a result, most laboratory personnel, regardless of their training and experience, are not familiar with the basic characteristics and natural histories of these agents. Additionally, lack of familiarization with the four levels of biosafety and chain of custody criteria represent potential barriers to the laboratory in responding to a bioterrorism event. Whether a bioterrorism incident is announced (overt) or unannounced (covert), the clinical laboratory may be requested to analyze human or environmental specimens or to forward them to a designated reference laboratory. In either case, it is important to coordinate all activities with the local and state health departments and the Federal Bureau of Investigation. A chain of custody document should accompany any specimen from the moment of collection to maintain documentation for any criminal investigation. Alternatively, the hospital laboratory may be consulted by physicians seeking information regarding the collection and transport of specimens suspected of harboring an agent of bioterrorism. As a first responder, the laboratory may be the first to encounter a biological agent. In these cases, the laboratory personnel should be familiar with what steps are to be taken to assist in the diagnosis of each disease.

To address the serious deficiencies in the current state of laboratory preparedness and to improve response, the CDC has created the Laboratory Response Network, which has been designed to provide an organized response system for the detection and diagnosis of biological agents based on laboratory capacity and the degree of risk. Four levels of laboratory capacity, referred to as biothreat (BT) levels, have been identified: BT levels A, B, C, and D. Each level has designated core testing capacities, including specific biosafety requirements. For example, a BT level A laboratory must have a certified biological safety cabinet, be capable of ruling out critical biological agents, and refer the agent or specimen to a higher-level laboratory, i.e., a BT level B facility. The most complex diagnostic testing, including archiving, agent identification and confirmation, and evaluation of unknown agents for synthetic molecular chimeras would be performed in a BT level D facility, which functions at the highest biosafety level (i.e., biosafety level 4). Therefore, the complexity in diagnostic testing and handling of biological agents increases with the level designation. Level A laboratories have the minimum core capacity, and the highest capacity and most advanced techniques are offered by level D laboratories. Most community, private, university, and commercial laboratories are classified as BT level A and operate at biosafety level 2.

As a first responder, the clinical laboratory will play a pivotal role in responding to an act of bioterrorism. Several issues must be addressed before the laboratory can effectively play its role as a first responder: (*a*) knowledge of the current biosafety level within the laboratory; (*b*) development and availability of protocols re-

lated to the chain of custody, collection, preservation, and shipment of specimens and cultures and detection and identification of targeted agents; (*c*) location of the nearest higher-level reference laboratory; (*d*) knowledge of current guidelines to ensure the safe handling and shipment of biological agents; and (*e*) knowledge of the basic characteristics (e.g., microscopic, cultural, and biochemical) of the current targeted agents. Until additional rapid and reliable detection systems and diagnostic tests are available, the clinical laboratory must rely on conventional methods to obtain as much information in the least amount of time to effectively provide the other members of the first-responder team (i.e., primary care practitioners, hospital emergency room staff, infectious disease practitioners, infection control practitioners, and administrators) with information concerning the nature of the agent. Such information is vital and essential to patient management; institution of focused infection control and prevention measures; protection of healthcare personnel, patients, and facilities; and the notification of local, regional, state, and federal agencies regarding the nature and type of bioterrorism event. Therefore, the major components of the clinical laboratory's role as a first responder include (*a*) awareness through active surveillance, (*b*) detection and characterization of unusual organisms, (*c*) rapid diagnosis and identification of the etiological agent, (*d*) preparedness (e.g., training of personnel, protocols, diagnostic testing procedures), (*e*) individual and collective safety, and (*f*) communication of information to local, regional, state, and federal officials.

ANTHRAX

Infection with *B. anthracis* (anthrax) can lead to septicemia, tissue necrosis, multiple organ failure, and death. The endospores of anthrax, a gram-positive bacillus found in the soil, are resistant to heat, drying, ultraviolet and gamma radiation, and many disinfectants. Endospores are produced when deleterious conditions exist; they can survive for decades in the environment and are adaptable to being aerosolized *(70, 71)*. Anthrax infection is considered a rare event, but it has been implicated in several outbreaks, including 25 cutaneous infections caused by a single cow in Paraguay in 1987 and thousands of infections in Zimbabwe in the early 1980s *(71)*. However, because of its propensity to be used as a weapon of disease and death, it has attracted much attention in recent years. Anthrax is the first bacterial disease for which immunization became available, in 1881.

Currently, the licensed vaccine is available for subcutaneous administration at 0, 2, and 4 weeks and 6, 12, and 18 months with yearly boosters thereafter *(72)*. The anthrax vaccine is derived from a purified culture filtrate of an avirulent strain of *B. anthracis* that has a high antigen content *(73)*. Reports of bioterrorism threats of exposure to anthrax in 1998 in Kentucky, Indiana, Tennessee, and California have raised the possibility of its use as a weapon in domestic terrorism *(74)*. In fact, a disturbing scenario reported by Inglesby *(75)* outlines the course of events in a possible scenario involving the release of anthrax during a football game in the Northeastern United States. Unfortunately, it is not until 3–4 days later, after the deaths of several people, that the agent is identified as anthrax. In this hypothetical situation, the involvement of the medical community, health department, and law enforcement units, the use of antibiotics, the massive rush of people seeking help or information, and the aftermath of such an attack are thought-provoking.

Exposure to anthrax can occur after contact with infected animals or humans via abrasions or through inhalation, ingestion, or contact with the skin *(70)*. When exposure is cutaneous, infection is generally curable and rarely fatal. Cases of gastrointestinal exposure, such as eating infected meat, are extremely rare. Inhalation exposure among slaughterhouse and textile workers is somewhat more frequent. However, this has been managed effectively by immunization. When *B. anthracis* is released in an aerosol form, the spores enter the pulmonary macrophages, which carry the organism to the lymph nodes and other suitable environments for its growth *(71)*. A capsule, various proteins, and toxins are produced by the organism; the toxin can cause septicemia, tissue necrosis, multiorgan failure, and death. Symptoms of anthrax infection include fever, malaise, cough, and respiratory distress; if untreated, shock and death can occur within 36 h *(72)*. The infection can be treated by intravenous administration of Ciprofloxacin (400 mg every 8–12 h), doxycycline (a 200-mg loading dose with 100 mg every 8–12 h), or penicillin (2 million units every 2 h) plus streptomycin (30 mg/kg per day intramuscularly) *(72)*. Antibiotics alone are effective only for treatment of susceptible organisms; however, a combination of antibiotic and vaccine therapy is more effective *(73)*. In fact, such a combination can reduce the 60-day antibiotic regimen by one-half, reducing antibiotic use and the potential for resistance. Laboratory diagnosis involves either an immunodiagnostic technique, using a direct fluorescent assay that detects the capsule after growth in 8 g/L sodium bicarbonate and CO_2, or culture on 50 mL/L sheep blood in agar at 35 °C for 18–24 h for detection of a gram-positive aerobic (nonhemolytic, nonmotile, and gammaphage-sensitive) organism that produces spores *(76)*.

Clear differentiation between *B. anthracis* and other members of the *B. cereus* group (i.e., *B. mycoides*, *B. cereus*, and *B. thuringiensis*) has been considered problematic in certain borderline isolates. This issue has led to reports of some anthrax cases as *B. cereus* or *B. anthrax similis (77)*. In practice, *B. anthracis* is identified by isolates that are nonmotile, sensitive to penicillin, have characteristic colonial morphology (on blood or nutrient agar), and are able to produce capsules in blood or nutrient medium containing 7 g/L bicarbonate after incubation in 5–20% CO_2 atmosphere. These isolates also have a matted ap-

pearance, look fairly flat, are markedly tacky, and are white or grayish-white in color *(77)*.

SMALLPOX

Similar to other poxviruses, smallpox (variola major) produces cutaneous vesicular eruptions *(78)*. Infection with this virus has a high mortality rate (25–30%) and is accompanied by a fever. Despite the official worldwide eradication of the virus nearly two decades ago, renewed interest in this virus is emerging because of its potential use in bioterrorism. An attack involving this virus is highly technical and could materialize mainly through the involvement of governments considering biological warfare *(79)*. Ken Alibek, a former director of the biological weapons programs in the former Soviet Union, has indicated that smallpox was stockpiled in Novosibirsk, Russia *(80)*. The last known cultures of smallpox are stored at the CDC in Atlanta and in the Novosibirsk Vector Facility *(80)*.

Smallpox is one of the most dangerous organisms known to humankind. It is transmitted through person-to-person contact via inhalation of air droplets or through dispersion as an aerosol *(81)*. Transmission can also occur through contact with contaminated articles (e.g., bedding and clothes) *(82)*. The incubation period of smallpox virus is 9–10 days after exposure, during which transmission of the virus to others can occur within minutes of contact *(80)*. Patients are most contagious late in the incubation period or when the fever appears; the oropharynx of the infected individual is the main source of transmission *(82)*. Smallpox and chickenpox are most often confused during the first 2–3 days of rash, except that smallpox lesions develop at a slower rate and are evenly distributed on the surface of the body, whereas chickenpox lesions develop in clusters and the rash is more intense *(81)*. Chickenpox lesions are not found on the palms or soles and are generally superficial. After 2–4 days of fever, antibodies against the virus developed by the host eliminate it from the blood; however, by this time, the virus has spread throughout the body, especially within the epidermal cells *(82)*.

Virus particles can be identified by negative-staining electron microscopy, and exposure can be established by quantifying the antibodies during the second half of the incubation period by hemagglutination inhibition, complement fixation, neutralization, and gel-precipitation techniques *(78)*. Intraepidermal vesicles in the skin and mucous membranes are considered the most dominant histologic features of smallpox *(82)*. Smears of cells from vesicles contain large eosinophilic cytoplasmic inclusion bodies, the detection of which can provide a simple test for smallpox *(82)*. Vaccination before or within the first 4 days after exposure can provide almost complete protection and prevent death *(81)*. The eradication of smallpox through immunization programs has been one of the major successes of medicine. A large supply of the vaccina virus vaccine is needed to launch a successful defense program against any bioterrorist attack involving smallpox *(73)*. Rapid identification of the virus and confirmation of exposure in patients can assist in timely and life-saving administration of the vaccine in potentially exposed individuals.

Although the risk of a biological attack is low, the question is no longer if such an event will occur, but rather when it will occur. In response to this threat, the medical community must be prepared to respond to a suspected or confirmed bioterrorism event quickly, efficiently, and in a safe manner, providing protection for healthcare personnel, patients, and those individuals who are not certain of their exposure but seek medical care because of panic and the need to receive prophylactic therapy, if available.

Summary

In response to chemical or biological attacks, clinical laboratories, as integral components of local medical teams, should be prepared to provide diagnostic, monitoring, and specimen-handling support. In the case of chemical warfare, rapid identification of the specific agents involved can be important in designing treatment strategies. This role becomes even more important when antidotal therapies for the suspected agents are available. In cases of biological terrorism, identification of the organisms or agents involved and proper handling of specimens to be sent to higher BT-level facilities are major tasks for the clinical laboratories. Providing educational material or seminars on various agents with the intent of raising awareness within a given institution is also an area in which laboratories should assume an active role. Because of the uncertainty and panic created by a suspected or confirmed act of bioterrorism, the healthcare delivery system may be overwhelmed by individuals seeking medical care; any response by the medical community must first deal with this problem. Therefore, the role of the clinical laboratory in the timely diagnosis and identification of organisms involved in bioterrorism is of paramount importance in the medical management of victims.

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