ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Review Article

Advancements in Biosurveillance: Detecting Infectious Biological Agents

Syeda Hina Shah[1], Aneeza Batool[1], Sabahat Fatima[1], Ammara Kausar[1], Imran Shahid[2], Sadia Aziz[3], Nazir Ahmed Lone[1], Vivek Sharma[4] and Liaqat Ali[1]*

[1]Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan

[2]Department of Pharmacology and Toxicology, Faculty of Medicine, Umm Al-Qura University, Al-Abidiyah, Makkah, Saudi Arabia

[3]Department of Biological Sciences, International Islamic University Islamabad, Pakistan

[4]Department of Environmental Sciences, University of Freiburg, Freiburg, Germany

***Corresponding Author:** Liaqat Ali, Assistant Professor and Head Section of Microbiology and Immunology, Department of Biological Sciences, National University of Medical Sciences (NUMS), Rawalpindi, Pakistan.

Received: December 16, 2024; **Published:** January 30, 2025

Abstract

Bioterrorism also called biological attack, refers to the deliberate release of microbes with the potential to cause illness or death in humans, animals, or crops. These weapons can trigger large-scale epidemics with unprecedented lethality and both nation-states and terrorist organizations have employed them for devastating consequences. Medical professionals recognize the critical importance of swiftly and accurately detecting acts of bioterrorism due to these bio-threat agents' unpredictable and destructive nature. Ensuring effective control of these bio-threat agents is of utmost importance, considering the difficulties in predicting and preempting their destructive consequences. A range of advanced testing methods such as immunological assays, molecular analysis, and bioluminescence analysis are currently accessible to detect biological threat agents precisely. In this context, we emphasize emerging technologies that are pivotal in enhancing the precision of bio-warfare agent detection, thus addressing previously formidable challenges.

Keywords: *Bioterrorism; Bio-Threats; Bioweapons; Bio-Detection Technologies; Outbreaks*

Abbreviations

ICT: Immunochromatography Test; ELISA: Enzyme-Linked Immunosorbent Assay; ECL: Electrochemiluminescence; PCR: Polymerase Chain Reaction; rRNA: Ribosomal RNA; RT-PCR: Reverse Transcription Polymerase Chain Reaction; CANARY: Cellular Analysis and Notification of Antigen Risks and Yields; FRET: Fluorescence Resonance Energy Transfer; MS: Mass Spectrometry; HPLC: High-Performance Liquid Chromatography; AFM: Atomic Force Microscopy

Highlights

- Bioterrorism is the deliberate release of harmful biological agents.
- Bio-threat agents can be difficult to predict and prevent.
- Early detection is crucial for its effective control.
- New technologies are improving the accuracy of bio-warfare agent detection.

Introduction

Bioterrorism involves the intentional release of biological agents or toxins that can cause harm to humans, crops, or animals on a large scale. This substantially threatens public health and national security and can negatively impact a nation's well-being, leading to significant disruptions in routine activities. A variety of pathogens and their toxins have been utilized in bioterrorism, including those of bacterial, viral, parasitic, or fungi [1]. The development of advanced detection techniques has led to rapid identification and response to bioterrorism. Pathogen identification is a crucial first defense against bioterrorism, and efforts have been made to establish fast, accurate, and sensitive assays for the diagnosis of infectious disease agents likely to be used in bioterrorism. This review article aims to emphasize the significant potential of biological warfare as a causative factor in bioterrorism and the role of advanced diagnostic methods in rapidly detecting biowarfare agents, ultimately helping mitigate bioterrorist attacks.

Background

After UN resolution 2162B (XXI) was enacted in 1967, condemning all activities adverse to the Geneva Protocol, the WHO formally recognized the threat of biological and chemical warfare. This culminated in the 1970 WHO study "Health Aspects of Chemical and Biological Weapons," which was modified in 2004 into WHO advice "Public Health Response to Biological and Chemical Weapons." This World Health Organization paper focuses on recognizing and reacting to unexpected illness outbreaks. Despite efforts to limit the use of biological weapons with the 1972 Conventions on the Use of Biological Weapons, there remains a concern, especially for future critical care physicians. The definition of bioterrorism varies from source to source and has evolved. In the 1990s, definitions mostly focused on bacterial or viral biological agents [2,3]. Figure 1 depicts the historical perspective of bioterrorism.

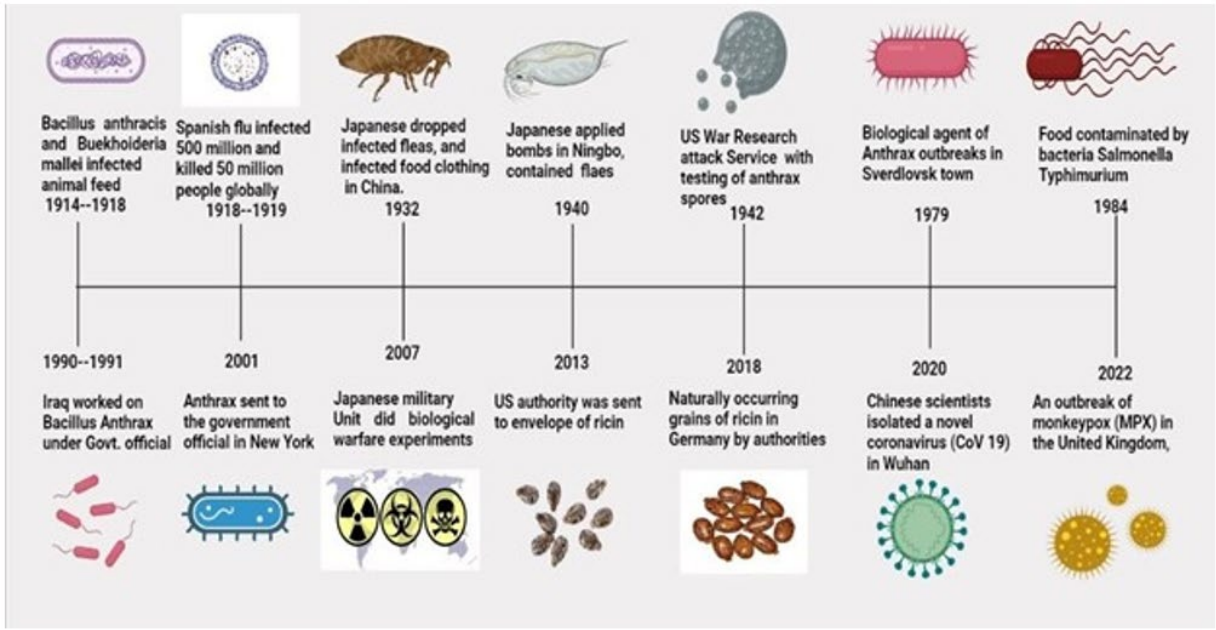

Figure 1: *Historical perspective of bioterrorism. This figure depicts a concise overview of various bioterrorist attacks that have occurred in the past (Image generated:* www.biorender.com*).*

Human pathogenic microbes involved in biowarfare

Human pathogenic microorganisms involved in bioterrorism are bacteria, viruses, fungi, and parasites are the main groups of human harmful microorganisms. These microbes lead to human pathogenic disorders varying from mild to serious life-threatening illnesses. The Disease Control and Preventive Center and the National Institute of Allergy and Infectious Diseases of Health have categorized biological agents into three priority groups as shown in table 1.

Category	Definition	Disease	Organism/s	References
A	High-priority agents are organisms that represent a threat to national security because they: can be readily spread or passed from person to person, have high mortality, and have the potential for significant public health impact can lead to public panic and societal unrest, and necessitate special action for public health awareness.	Anthrax	*Bacillus anthracis*	[28-33]
		Botulism	*Clostridium botulinum toxin*	
		Plague	*Yersinia pestis*	
		Smallpox	*Variola major*	
		Viral hemorrhagic fever	*Filoviruses (Ebola Marburg)* *Arenaviruses (Lassa, Machupo)*	
		Brucellosis	*Brucella species*	
		Food safety threats	*Salmonella species* *Shigella* *Escherichia coli 0157:H7*	
B	The second highest priority agents are those that: are reasonably easy to disperse and result in moderate morbidity and fatality rates. Specific improvements in laboratory diagnosis capabilities and illness surveillance are required.	Q fever	*Coxiella burnetiid*	[28,34-38]
		Ricin toxin	*Ricinus communis (castor beans)*	
		Staphylococcal enterotoxin B	*Staphylococcus aureus*	
		Typhus fever	*Rickettsia prowazekii*	
		Viral encephalitis Joint swelling or rash	*Alphaviruses (Venezuelan, equine encephalitis, eastern equine encephalitis, western equine encephalitis)* *Chikungunya virus*	
		Water safety threats	*Vibrio cholera* *Cryptosporidium parvum*	
C	The third highest priority agents are Emerging pathogens, which might be developed for mass dispersion in the future and have ease of availability, production, and distribution. High morbidity and mortality risk, as well as significant health consequences.	Emerging infectious diseases	*Nipah virus* *Hantavirus* *Tick-borne hemorrhagic fever viruses* *Tick-borne encephalitis viruses* *Yellow fever* *Multidrug-resistant tuberculosis*	[34-38]

Table 1: *Categorization of potential biological agents responsible for bioterrorism.*

Rapid detection of biological agents by advanced technologies

Early pathogen detection plays a defensive role against bioterrorism. These advanced technologies are valuable for diagnostic purposes and offer beneficial insights into outbreaks.

Serological assays

Antibodies in the blood can be detected and their concentration measured using a serological test. It is an essential tool for many kinds of medical and health-related research [4]. Serological assays play a critical role in the identification and detection of potential biowarfare agents, including mycotoxins, saxitoxin, ricin, cholera toxin, *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis*. These assays exhibit a high degree of specificity and can detect harmful biological substances across a range of biological samples.

Immunochromatography test ICT

Immunochromatography, a rapid screening assay also known as lateral flow assay, utilizes chromatography and immunoassay techniques to detect the presence of specific target substances or biological markers in a sample. Immunochromatography offers rapid, durable, economical, and accessible results for accurate diagnosis. However, it has certain limitations like lower sensitivity, subjective interpretation, and uncertainty about the viral antigen source [5].

Enzyme-linked immunosorbent assay (ELISA)

ELISA is utilized to identify mycotoxins in contaminated food and feed. Similarly, monoclonal antibodies are employed to detect saxitoxin, a potent marine toxin. Detection methods such as immunodiffusion and ELISA are utilized for ricin—a toxin derived from castor beans. ELISA is an immunological assay that works on the principle of antigen-antibody interactions and determines the concentration of targeted antigen or antibody in the sample. For test result validation, it will need to perform a western blot using target-specific antibodies or an immune-fluorescent antibody [6].

Electrochemiluminescence ECL

Electrochemiluminescence (ECL) is a technique used to detect and quantify biological molecules based on their ability to produce light through a redox reaction. The principle of this technique is based on luminophores. They attain a high-energy state through an oxidation-reduction process, causing electrons to move across the electrode's surface. Upon returning to the ground state, the stimulated luminophores release light in the form of photons [7]. By labeling biomolecules with luminophores and measuring the quantity of light they produce, it becomes possible to identify and measure these molecules shown in figure 2.

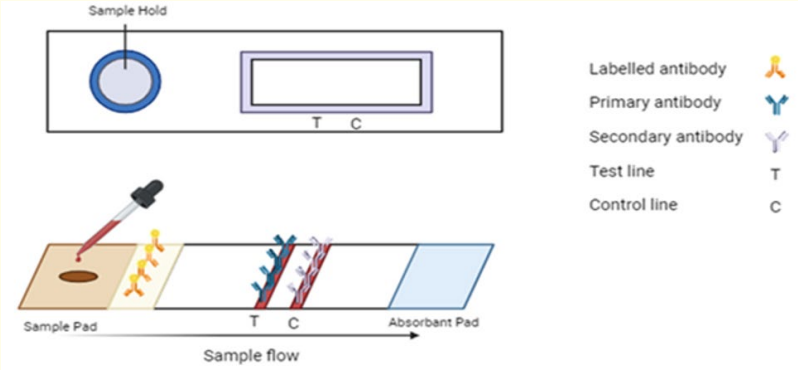

Figure 2: *The Immunochromatography Test (ICT) provides quick, on-the-spot diagnostics by identifying target antigens using specialized antibodies—a smooth combination of precision and speed transforming healthcare screening (Image generated:* www.biorender.com*).*

ECL is highly sensitive and used in clinical chemistry, environmental analysis, and the study of proteins, DNA, and viruses, making it valuable for the detection of biowarfare agents. It offers minimal interference and high sensitivity due to multiple excitation cycles, providing a wide range of detection spectrum [8,9]. However, ECL is susceptible to light leaks and background luminescence, requires pure reagents and solvents, and may lead to pulse pileup due to the accumulation of bright flashes of light, potentially underestimating the actual light intensity.

Molecular approaches

The genetic analysis of biowarfare agents involves utilizing the DNA within the genomes of microbial strains to amplify pathogen-specific nucleic acid fragments or detect unique gene sequences through hybridization with complementary nucleic acid probes [10]. These approaches are based on the genomic variations present in microorganisms, providing a valuable method for verifying the presence of biowarfare agents.

Polymerase chain reaction (PCR)-based amplification

Polymerase chain reaction (PCR)-based amplification is the most effective strategy for pathogen identification. The nucleic acid-based approach for bacterial, fungal, and viral pathogenic agents is recognized by using highly conserved ribosomal RNA (rRNA) genes, intergenic sequences, and especially toxin genes [11]. Reverse transcription polymerase chain reaction (RT-PCR) is a sensitive technique in which cDNA from target RNA is synthesized for subsequent PCR reactions and then quantified. This approach has been used to detect Aspergillus (a fungal pathogen) in household and hospital water, and *Cryptosporidium parvum* oocysts (an intestinal pathogen) in municipally treated water.

PCR is helpful in clinical and public health laboratories for potent diagnostic tests. Moreover, it is a quick, sensitive, and specific method. Man-made occurrences of a deadly toxin gene (botulinum toxin) in a recipient nonpathogenic organism such as *Escherichia coli* or *Bacillus subtilis* can also be detected by employing this technique [11].

Real-time probes

In this technique, specific primers are used to amplify the target DNA or RNA sequences and for detection intercalating dyes are used. Probe-target hybridization is a temperature-sensitive approach. It relies on the composition of nucleotide probes. The high GC contents are highly prone to non-specific primer annealing. However, this problem can be overcome by using fluorescently labeled probes such as the 50 endonuclease, adjacent linear, and hairpin oligo-probes.

In the real-time assay, other probing systems are widely used including LightCycler™, TaqMan™, and Molecular Beacons. The LightCycler system quantifies the fluorescence resonance energy transfer (FRET) between two linear oligonucleotide fluorophore-labeled probes. The probes bind specifically to the target in a head-to-tail motif, leading to energy transfer in the form of fluorescent light emission. The TaqMan probe-based approach is designed in which both primer and probes anneal to their complementary region on the template DNA. Each probe is labeled with a specific reporter dye, allowing the detection and discrimination of the multiple PCR products produced by various sets of primers in a single reaction [12]. Molecular Beacons are small, single-stranded nucleic acid hairpin fluorescent-labeled probes that hybridize to target sequences specifically. A fluorophore hybridizes with one end of the stem sequence and along with a quencher binds to the other end.

Molecular Beacons are better than most linear probes to monitor amplicons accurately in PCR reactions because a single nucleotide mismatch can prevent a Molecular Beacon from binding to its target and producing fluorescence. Both TaqMan and Molecular Beacons can detect single nucleotide changes and are highly suitable for allele-specific discrimination.

Multiplex assays

A single multiplex assay that incorporates multiple probes labeled with a fluorophore and thus can detect numerous pathogens in a single reaction tube. It is a cost-effective technique in the clinical microbiology laboratory. This assay can improve the efficiency of biological toxin detection [6]. The novelty of this assay is that it can target more than 50 toxins simultaneously in a single reaction.

DNA microarrays

Microarrays of nucleic acids allow thousands of targets to be analyzed simultaneously, which is especially useful for novel variants identification and characterization. Gene fragments will be cloned in long (70-80mer) oligonucleotide segments that are glued to a glass slide or other solid matrix, like those used for computer chips. This technique can be performed by interrogating many genes using a DNA microarray format with smaller yet specialized sequences. Each array is typically made up of a sequence of known 70-basepair oligonucleotide targets linked to solid platforms [10].

Biosensors assays

Recently, for rapid pathogen detection biosensor systems have been used. The sensitive system called CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) utilizes B-lymphocytes genetically engineered to express both cytosolic aequorin, a calcium-sensitive bioluminescent protein, and membrane-bound antibodies specific for a given pathogen or toxin. Interactions of antigens with antibodies elevate intracellular calcium levels resulting in light emission by the cytosolic aquorin molecules. This technique is more rapid, sensitive, and specific than most antigen detection systems and has been shown to detect *Yersinia pestis* in less than 3 min at levels of 50 colony-forming units.

Cell-based sensors

The application of cell-based methods for biothreat agent identification has witnessed remarkable advancements during the last decade. Cell-based sensors can be divided into two categories [13]:

- **Innate cell-based sensors:** Several sensing systems based on cell's innate physiological responses to biothreat have been developed recently. For signal transduction, one kind focuses on monitoring the electrical excitability of the mammalian cell membrane in response to biothreat analyte. It monitors a wide range of possible poisons using a neuroblastoma-glioma cell line [13].
- **Engineered cell-based sensors:** Synthetic biological systems called engineered cell-based sensors have been developed to recognize certain substances or signals in their surroundings. Frequently, these sensors rely on genetic circuits and cellular apparatus to provide a quantifiable result upon encountering the intended chemical or signal. The development and use of biological systems that can identify and react to certain threats is a necessary step in the development of modified cell-based sensors for the detection of bioterrorist agents. These sensors frequently depend on cells' capacity to recognize and react to certain chemicals or compounds linked to bioterrorist agents [14].

Hybrid technologies

Hybrid technologies are commonly employed to detect potential biowarfare agents such as *Yersinia pestis, Francisella tularensis, Bacillus anthracis, Vibrio cholera, Ricin, Staphylococcal enterotoxin B, Botulinum toxin, and Brucella spp*. These integrated technologies significantly improve the sensitivity, selectivity, and precision of the analysis, serving as a robust toolkit for detecting and characterizing specific biomarkers associated with these agents.

Mass spectrometry

Mass spectrometry (MS) plays a pivotal role in bioterrorism detection by enabling the rapid and accurate identification of potential bioterrorism agents. It offers the capability to detect and analyze a wide range of biomolecules associated with bioterrorism agents, with high sensitivity and specificity. This technique relies on an ion source, a detector, and a mass analyzer, all operating within a high vacuum environment, presenting a fundamental configuration shared by all mass spectrometers [15]. For the detection in mass spectrometry, a sample is prepared using chromatography techniques, to facilitate the detection of bioterrorism agents in the gaseous or liquid phase. This sample preparation process aims to eliminate interferences, concentrate the analyte, and convert it for detection or separation, thus preventing endogenous compounds and co-eluted products from impacting the performance of mass spectrometry [16].

Gas chromatography

Gas chromatography is a technique used to separate components of a gas mixture based on their physical characteristics, such as shape, size, molecular weight, and boiling point. This method can be utilized to vaporize and separate volatile components from a sample, making them suitable for analysis in a mass spectrometer [17].

Liquid chromatography

Liquid chromatography separates samples based on their interactions with stationary and mobile phases, often influenced by polarities. This method can be employed to separate components in a sample, allowing for further detailed analysis of each component. The separated components can then be examined in more detail using mass spectrometry, aiding in the detection and identification of potential bioterrorism agents [18].

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is a separation technique based on the interactions between sample components, a stationary phase, and a mobile phase. Figure 3 illustrates the HPLC method, which pumps a liquid sample through a chromatographic column, enabling the separation of components based on their binding affinities to the stationary phase. Typically employing a UV-Vis detector for analysis, HPLC produces a chromatogram that provides valuable insights into the composition of the sample [19,20].

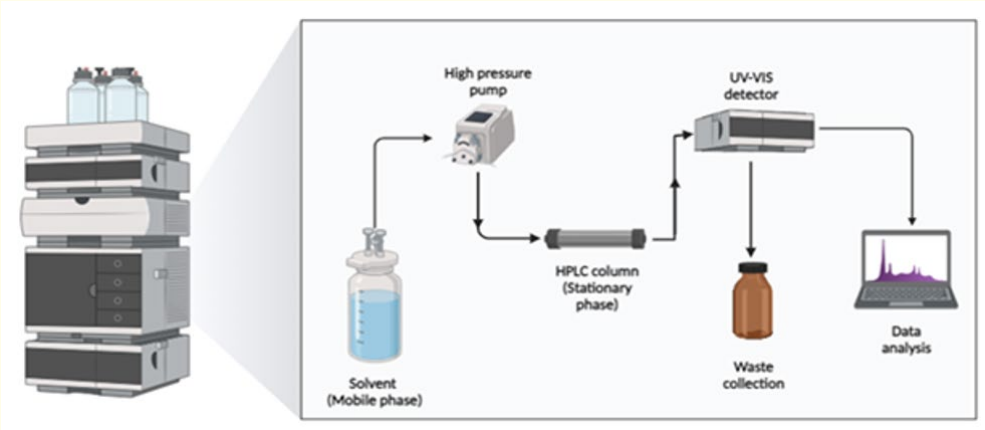

Figure 3: *Diagrammatic representation of precise chemical separation as performed by HPLC. HPLC is a fundamental tool in scientific study that helps to separate the complex structure of various substances by utilizing sophisticated chromatographic procedures (Image generated:* www.biorender.com*).*

Atomic force microscopy

Atomic Force Microscopy (AFM) is a high-resolution scanning probe microscopy technique that produces images of surfaces at the atomic or near-atomic level. AFM is a technique that use of the atomic force between the probe and the surface of the sample. It is a flexible tool that can function in liquid, high vacuum, and ambient air settings, making it a perfect imaging approach for biomedical research. AFM can photograph biological samples in buffer solutions, which is advantageous for preserving specimens in their actual conditions. Secondly, an AFM may acquire 3D topography data in its simplest form. A tiny probe with a particularly sharp tip and a cantilever is used in AFM to contact the sample surface [21]. The topography of the sample causes the tip to be deflected as it passes across the surface. A laser beam that bounces off the cantilever is used to measure this deflection, which enables the mapping of the surface's topography (Figure 4).

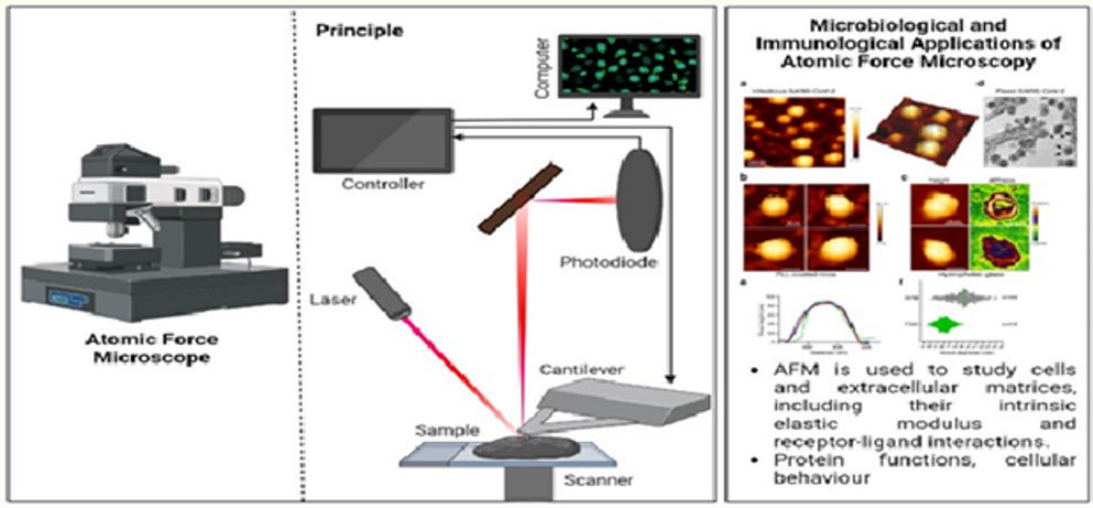

Figure 4: *Atomic force microscopy (AFM) uses an acute tip to scan surfaces with extreme precision; observing at the nanoscale. Discover the mysteries of molecular landscapes as AFM reveals the complex realm of nanotechnology (Image generated:* www.biorender.com*).*

AFM has certain limitations like AFM imaging can be slow compared to other microscopy techniques. Depending on the size of the sample and the desired resolution, the time needed to scan a sample at high resolution might vary from minutes to hours. Biological samples frequently require preparation processes, such as coating with a thin layer of metal or immobilization on a flat surface. This can cause distortions in AFM measurement [22].

Biowarfare agents and their advanced detection techniques are presented in table 2.

Discussion and Conclusion

The use of biological toxins in bioterrorism has become a universal and widespread problem. It has opened the discussion to address global security and the need for strong measures to combat the potential use of biological toxins. These biotoxins can initiate large-scale epidemics with unparalleled lethality resulting in mass casualties and disruption of society [1].

Method	Detection techniques	Biowarfare agents
Serological assays	Enzyme-linked-immunosorbent assay ELISA	*Mycotoxins* *Saxitoxin* *Ricin* *Cholera toxin* *Bacillus anthracis* *Yersinia pestis* *Francisella tularensis* *Shigella spp.* *Smallpox* *Influenza* *Prions* *Avian influenza* *Mycobacterium tuberculosis* *Hemorrhagic fever viruses (Ebola, Norovirus, Marburg)*
	Immunochromatography test ICT	
	Electrochemiluminescence ECL	
Immunological probes	Antibody-based-probes (immunosensors)	Toxins produced by: • *Bacillus anthracis* • *Clostridium botulinum* • *Staphylococcus aureus*
	Ligand-based probes	
Genomic assays	Polymerase chain reaction PCR	*Bacillus anthracis* *Yersinia pestis* *Shigella spp.* *Smallpox* *Francisella tularensis* *Hemorrhagic fever viruses* *Influenza* *Norwalk virus* *Avian influenza* *Mycobacterium tuberculosis* *Parasitic protozoa*
	Real-time Probes	
	Microarray	
	Multiplex assay	
	Fluorescence-based oligonucleotide detection system	
Hybrid technologies	Mass spectrometry MS	*Yersinia pestis* *Francisella tularensis* *Bacillus anthracis* *Vibrio cholera* *Ricin* *Staphylococcal enterotoxin B* *Botulinum toxin* *Brucella spp.*
	Gas chromatography GC	
	High-performance-liquid chromatography HPLC	
	Antigen capture chromatography	

Table 2: *Methods employed in the detection of various biological warfare agents.*

This review provides a brief overview of the historical use of bioterrorism to inflict terror in society; followed by comprehensive details on the potential use of biotoxins in bioterrorism and the associated public health implications. It covers a wide range of biotoxins, including bacterial pathogens, as well as viral pathogens. These biological warfare agents have unique properties, pathways of transmission, and

potential adverse effects on human and animal health. Moreover, this review has provided insight into many conventional and newly developed techniques for diagnosing bio-warfare agents. Each of these detection methods is associated with a specific biological warfare agent for accurate identification and ultimately helping to combat it.

Reducing the impact of bioterrorism on human life requires the development of early detection methods to minimize their impact. Early diagnosis including identification of microbes, antimicrobial therapeutics, public awareness, and training will enhance the overall strength of a society to fight outbreaks of infectious diseases and help alleviate the effects of bioterrorist attacks. This comprehensive discussion of biological warfare agents and their detection methods highlights the importance of continuous research and development in this field.

Limitations and Prospects

The use of specific diagnostic tests for the identification of specific biowarfare agents is a major limitation of various assays. Their detection limits, specificity, and sensitivity vary, making it unclear for the technologist to select a single technique. The current review just focuses on BioWare agents but needs to study chemical toxic agents which may also contribute to destroying a community. Continuous research for the development of different diagnostic assays for bioterrorism preparedness will tend to improve diagnostic tools, more effective countermeasures, and enhanced surveillance systems. Furthermore, the incorporation of artificial intelligence and machine learning algorithms can enhance the threat detection and response system.

My heartfelt gratitude goes to Dr. Liaqat Ali, Virologist, National University of Medical Sciences (NUMS), Rawalpindi, for giving personal advice, assistance, and facilitation from the start of this review to the end. His assistance provided insight and wisdom to my thinking. During the study period, his loving demeanor was highly encouraging and motivating.

Funding Support

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Financial Interests

The authors declare that there were no material/financial interests that relate to the research described in this paper.

Authors' Contribution

LA supervised and conceived the study. All authors contributed equally to study design, writing, and editing. All authors reviewed and agreed with the final version of the manuscript.

Conflict of Interest

The authors state that there are no conflicts of interest in this work.

Bibliography

1. Rathish B., *et al*. "Comprehensive review of bioterrorism". In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing (2023).
2. Clark DP and Pazdernik NJ. "Biological warfare: Infectious disease and bioterrorism". *Biotechnology* 24 (2016): 687-719.
3. Ns E., *et al*. "Zoonotic and parasitic agents in bioterrorism". *Journal of Infectious Diseases and Travel Medicine* 4.2 (2020): 1-7.

4. Metropolis India Lab [Internet]. Serological Test: Serological Test: Overview, Differences, Types And Uses | Metropolis TruHealth Blog (2024).

5. Lim GS., *et al*. "Chemiluminometric immunosensor for high-sensitivity cardiac troponin I employing a polymerized enzyme conjugate as a tracer". *Scientific Reports* 5.1 (2015): 14848.

6. Perlin D. "Rapid detection of bioterrorism pathogens". *Beyond Anthrax* 10 (2008): 317-334.

7. Brown K., *et al*. "Electrochemiluminescence within veterinary Science: A review". *Bioelectrochemistry* 146 (2022): 108156.

8. Calabria D., *et al*. "Smartphone-based 3D-printed electrochemiluminescence enzyme biosensor for reagentless glucose quantification in real matrices". *Biosensors and Bioelectronics* 227 (2023): 115146.

9. Huang J., *et al*. "A novel electrochemiluminescence aptasensor based on copper-gold bimetallic nanoparticles and its applications". *Biosensors and Bioelectronics* 194 (2021): 113601.

10. Parida MM., *et al*. "Advance detection technologies for select biothreat agents". Handbook on Biological Warfare Preparedness (2020): 83-102.

11. Institute of Medicine (US) Committee on R&D Needs for Improving Civilian Medical Response to Chemical and Biological Terrorism Incidents. Chemical and Biological Terrorism: Research and Development to Improve Civilian Medical Response. Washington (DC): National Academies Press (US) (1999).

12. TaqMan multiplex qPCR for detecting animal species in meat and meat products: Development, recent advances and future p… (2024).

13. Kim E. Sapsford., *et al*. "Sensors for detecting biological agents". *Materials Today* 11.3 (2024): 38-49.

14. Kannappan S and Ramisetty BCM. "Engineered whole-cell-based biosensors: sensing environmental heavy metal pollutants in water—a review". *Applied Biochemistry and Biotechnology* 194.4 (2022): 1814-1840.

15. Lehmann WD and Jurgen H. "Gross: Mass spectrometry--A Textbook, 2nd edition". *Analytical and Bioanalytical Chemistry* 401.10 (2011): 3033-3035.

16. Bourgogne E and Wagner M. "[Sample preparation and bioanalysis in mass spectrometry]". *Annales de Biologie Clinique (Paris)* 73.1 (2015): 11-23.

17. Zhu J., *et al*. "Characterization of the key aroma compounds in Laoshan green teas by application of odour activity value (OAV), gas chromatography-mass spectrometry-olfactometry (GC-MS-O) and comprehensive two-dimensional gas chromatography mass spectrometry (GC × GC-qMS)". *Food Chemistry* 339 (2021): 128136.

18. Rappold BA. "Review of the use of liquid chromatography-tandem mass spectrometry in clinical laboratories: part II-operations". *Annals of Laboratory Medicine* 42.5 (2022): 531-557.

19. Lee TD. "Introduction to modern liquid chromatography, third edition". *Journal of the American Society for Mass Spectrometry* 22.1 (2011): 196-196.

20. ebin.pub. Principles of Instrumental Analysis [7andnbsp ed.] 9781305577213 (2023).

21. Lostao A., *et al*. "Recent advances in sensing the inter-biomolecular interactions at the nanoscale - A comprehensive review of AFM-based force spectroscopy". *International Journal of Biological Macromolecules* 238 (2023): 124089.

22. Malenica M., *et al*. "Perspectives of microscopy methods for morphology characterisation of extracellular vesicles from human biofluids". *Biomedicines* 9.6 (2021): 603.

23. Simonsen KA and Chatterjee K. "Anthrax". In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing (2024).

24. CDC. Centers for Disease Control and Prevention. Plague home | CDC (2021).

25. CDC. Centers for Disease Control and Prevention. Ecology and transmission of plague | CDC (2019).

26. Christian MD. "Biowarfare and bioterrorism". *Critical Care Clinics* 29.3 (2013): 717-756.

27. Mayo Clinic. Smallpox - Symptoms and causes (2024).

28. Riedel S. "Edward Jenner and the history of smallpox and vaccination". *Proceedings: Baylor University Medical Center* 18.1 (2005): 21-25.

29. Kagawa FT., *et al*. "Q fever as a biological weapon". *Seminars in Respiratory Infections* 18.3 (2003): 183-195.

30. CDC | Facts About Ricin (2019).

31. Fries BC and Varshney AK. "Bacterial Toxins-Staphylococcal Enterotoxin B". *Microbiology Spectrum* 1.2 (2013).

32. Services D of Hand H. Viral encephalitis. Department of Health and Human Services (2024).

33. Rahman Z., *et al*. "Vibrio cholerae transmits through water among the household contacts of cholera patients in cholera endemic coastal villages of Bangladesh, 2015-2016 (CHoBI7 Trial)". *Frontiers in Public Health* 6 (2018): 238.

34. Thavaselvam D and Vijayaraghavan R. "Biological warfare agents". *Journal of Pharmacy and Bioallied Sciences* 2.3 (2010): 179-188.

35. Alam AM. "Nipah virus, an emerging zoonotic disease causing fatal encephalitis". *Clinical Medicine (London)* 22.4 (2022): 348-352.

36. Hashmi HJ., *et al*. "Emerging epidemic of drug resistant tuberculosis in vulnerable populations of developing countries". *African Health Sciences* 17.2 (2017): 599-602.

37. Hecht G., *et al*. "Detection of Hantavirus during the COVID-19 Pandemic, Arizona, USA, 2020". *Emerging Infectious Diseases Journal* 29.8 (2023).

38. Holding M., *et al*. "Tick-Borne Encephalitis Virus, United Kingdom". *Emerging Infectious Diseases* 26.1 (2020): 90-96.

Volume 21 Issue 2 February 2025