Chapter 3
Nanomaterials-Based (Bio)Sensing Systems for Safety and Security Applications

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Abstract The development of new nanomaterials and nanotechnologies has provided many new opportunities for (bio)sensing systems. The introduction of nanomaterials, such as magnetic nanoparticles, gold nanoparticles, graphene, quantum dots, etc., is bringing advantages in terms of improving the selectivity and sensitivity of these systems. These nanomaterials also offer advantages in biosensors owing to their nanometric size, shape, composition, physical properties, ability to manipulate their surface chemistry and the property that they have in terms of adsorbing biological molecules and the change of their physical properties. In recent years, several bacterial pathogens, toxins, viruses, parasites and explosives have been considered as potential threats for bioterrorism, among which can find Escherichia coli, Salmonella, Bacillus anthracis, Clostridium botulinum, Botulinum Neurotoxin, Vaccinia, Plasmodium falciparum, Trinitrotoluene, etc. Bioterrorism is extremely complex to tackle but the science and technology are fundamental elements to reduce its threat. For this reason, monitoring systems for quick identification of biomolecules are the core of much of the basic research activities in combating bioterrorism. In this chapter we discuss the research efforts by using nanobiotechnologies with the aim of developing accurate, easy, cheap, portable and ultrasensitive assays for
agents that pose a biologic threat. Some nanomaterial-based (bio)sensing systems used to detect agents related with bioterrorism for safety and security applications in agriculture, food, forensic, biomedical are also given.

**Keywords** Nanomaterials • Magnetic nanoparticles • Gold nanoparticles • Graphene • Quantum dots • Nanotechnology • (Bio)sensing • Bacteria • Spores • Toxins • Virus • Parasites • Explosives • Bioterrorism • Biosystems

### 3.1 General Introduction. Nanomaterials and Bioterrorism

The discovery and study of nanomaterials has enabled the development of ultrasensitive (bio)sensing systems. This is due to their high surface area, favorable electronic and optical properties and electrocatalytic activity as well as good biocompatibility induced by nanometer size and specific physicochemical characteristics [1–5].

In recent years, the advent of nanomaterial-based (bio)sensing systems for safety and security applications is offering key researches and developments. In this context, of special interest are the ‘nanosized’ and nanomaterials based biosensors, called also nanobiosensors – a modern and efficient class of detection systems [1, 3, 6–10]. The application of these devices in food industry, environmental monitoring and clinic diagnostics could lead great improvements in safety and security against bioterror agents. Nowadays, laboratories and institutions related with the nano-biotechnology are working together to increase the capabilities to detect and respond to an attack by biological or chemical weapons [11].

Examples of nanomaterial-based bioterrosist agents for safety and security applications are given in the Table 3.1. The magnetic nanoparticles (M NPs), graphene (G), quantum dots (QDs), and more extensively gold nanoparticles (Au NPs) are being invaluable nanomaterials to detect bioterrorist analytes in macro- to nano-scale, including bacterial pathogens, toxins, viruses, parasites, explosives, etc.

Biological agents such as bacteria, viruses, biological toxins, and genetically altered organisms are contagious, and during this lag time, infected persons could continue to spread the disease, further increasing its reach. Hundreds or even thousands of people could become sick or die if a biological attack were to occur in a major metropolitan area [11]. For this reason, problems related to the risks of human health and environmental need to be carefully considered, mainly assays that involve a variety of safety and security applications.

In this chapter will be given some important strategies used in (bio)sensing systems with different nanomaterials to detect bioterrorist agents. In addition, this chapter will give opinions about the importance that these systems have for safety and security applications in food, environmental and other related fields.
| Agent | Analyte       | Nanomaterial                              | Technique      | Sample        | LOD            | Ref. |
|-------|---------------|-------------------------------------------|----------------|--------------|----------------|-----|
| Bacteria & Escherichia coli O157:H7 | Au NPs (13 nm diameter) | Electrochemical                            | Milk           | 50 CFU/strip  | [12]            |
|       | Cu@Au NPs (ca. 15 nm) | Electrochemical                            | Surface water  | 3 CFU/10 mL  | [13]            |
|       | Glyco-NPs      | Optical                                   | PBS            | 10⁴ cells/mL | [14]            |
|       | Silica particles (200 nm) coated with silver shells (ca. 20 nm) | Optical | Water | 3–5 cells | [15] |
|       | Salmonella spp. | Au NPs (ca. 25 nm) | Electrochemical | Pork | 1.0 × 10^2 CFU/mL | [16] |
|       | Salmonella typhi | Au NPs (ca. 15 nm) | Electrochemical | PBS | 98.9 CFU/mL | [17] |
| Spores | Bacillus anthracis | EAM | Electrochemical | DNA | 0.01 ng/μL | [18] |
| Toxins | Anthrax | Eu⁺ NPs | Optical | PBS | 10 pg/mL | [19] |
|       | Botulinum Neurotoxin B | Colloidal gold (25 nm in diameter) | IC | PBS | 50 ng/mL | [20] |
|       | Botulinum neurotoxin D | Colloidal gold (40 nm in diameter) | IC | Horse faeces | 50 ng/L | [21] |
|       | Botulinum neurotoxin A | QDs (655 nm) | Fluorescence | PBS | 5 pM | [22] |
| Viruses | H9 AIVs | Colloidal gold | IC | Chicken eggs | 0.25 units of HA | [23] |
|        | H1N1 flu | Colloidal gold | Electrochemical | PBS | 577 pM L⁻¹ | [24] |
| Parasites | Plasmodium falciparum | M NPs (1 μm diameter) | Electrochemical | Spiked serum | 0.36 ng mL⁻¹ | [25] |
|        | Au NPs | Electrochemical | Serum | – | [26] |
| Explosives | TNT | CdTe QDs | Fluorescence | Soil | 1.1 nM | [27] |
|        | 2.4-DNT | Graphene | Electrochemical | 0.5 M NaCl solution | 0.5 ppb | [28] |

PBS phosphate buffer solution, EAM electrically active magnetic nanoparticles, QDs quantum dots, M NPs magnetic nanoparticles, 2.4-DNT 2,4-Dinitrotoluene, TNT trinitrotoluene, Cu@Au NPs gold nanoparticles coated Cu, CFU colony forming units, NPs nanoparticles, CdTe QD cadmium telluride quantum dot nanoparticle, H9 AIVs H9 subtype avian influenza viruses, H1N1 flu Influenza A, IC Immunochromatography, LOD limit of detection, Eu⁺ NPs Europium nanoparticles
3.2 Monitoring Systems for Bioterrorist Agents

Numerous laboratories and public and private research institutions during decades have been designing and developing technologies to safeguard from biological attacks provoked by terrorists. Nowadays, they are developing monitoring technologies for bioterrorism agents with fast analysis, low limit of detection, and high accuracy to prevent false negatives and positives and maintain confidence in the monitoring system [30].

Among the highest-risk threat agents in bioterrorism can be considered: (1) Bacteria, they are prokaryotes, and their distribution is ubiquitous (humans, animals, and environment). They are the oldest living organisms in the history of this planet and play an important role in maintaining the ecosystem [11]. *Escherichia coli* (*E. coli*) is a family of naturally occurring bacteria. Some of these cause sickness and even death. Most *E. coli* infections come from eating undercooked (i.e. contaminated ground beef). Terrorists could use *E. coli* 0157:H7 bacteria as a weapon to strike many people at one time, also the *Bacillus anthracis* (*B. anthracis*) is considered a high pathogenic agent that could deliberate contamination events causing anthrax [31]; (2) Neurotoxins produced by *Clostridium botulinum* are among the most known poisonous substances [32]. For example, *botulinum* toxin is the most toxic substance known and is extremely poisonous by the oral route (estimated lethal oral dose, 10–70 μg for a 70 kg human) and potentially toxic by inhalation [33], for this reason it is used as bioterrorism agent (3) *Influenza A* (H1N1) *virus* also could be considered a bioterrorist agent, it belongs to the *Orthomyxoviridae* family and corresponds to the specific combination of glycol-protein hemagglutinin (HA) and neuraminidase (NA) variants, which are present on the surface of the enveloped RNA virus [24]. (4) Malaria is the most prevalent parasitic disease in the world caused by the apicomplex protozoan of the *Plasmodium* genus. Malaria is present over the tropics, where four species, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* are transmitted to humans by the bites of the female mosquito vector of the *Anopheles* genus [34] and although the use of parasites as bioterrorism agents has not received so much attention. Parasites could contribute to the installation of fear in human population upon intentional addition to their food and water supplies, which makes malaria suitable for being used by terrorists (5).

In recent years, the detection and quantification of nitroaromatic explosives such as 2,4,6-trinitrotoluene (TNT) have also received considerable attention due to their environmental, security against terrorists and health related concerns [35, 36].

Various methods have been developed for the detection of these bioterrorist agents and many more still are in development phase. Most of the assays are based on detecting the (a) whole organism, (b) bacterial antigens, and/or (c) the related nucleic acid. In the Table 3.2 are described some detection methods more commonly used for bioterrorist agents with safety and security applications.

Conventional methods such as the culture and colony counting methods that involve counting of bacteria, immunology-based methods that use antigen-antibody
| Bioterrorist agents | Detection methods | References |
|---------------------|-------------------|------------|
| **Pathogens**       | Culture and colony counting | [37–41] |
|                     | Immunology-based methods |            |
|                     | Polymerase chain reaction (PCR) |            |
|                     | Lateral flow |            |
|                     | Enzyme-linked immunoassays |            |
|                     | Biosensors |            |
|                     | Fluorescence immunoassay |            |
|                     | Chemiluminescence assay |            |
|                     | Electrochemical immunoassay |            |
|                     | Surface plasmon resonance sensor |            |
|                     | Fiber optic sensor |            |
|                     | Microfluidic biochip |            |
| **Viruses**         | Cell culturing | [42–44] |
|                     | Polymerase chain reaction (PCR) |            |
|                     | Enzyme linked immunosorbent assay (ELISA) |            |
|                     | Flow cytometry |            |
|                     | Lateral flow |            |
|                     | Biosensors |            |
| **Explosives**      | Fluorescence | [27, 45] |
|                     | Raman and mass spectroscopy |            |
|                     | Nuclear magnetic resonance spectroscopy |            |
|                     | Surface plasmon resonance spectroscopy |            |
|                     | Electrochemistry |            |
|                     | High performance liquid chromatography (HPLC) |            |
|                     | Gas chromatography–mass spectrometry (GC–MS) |            |
|                     | Electrogenerated chemiluminescence (ECL) |            |
|                     | Enzyme-linked immunosorbent assays (ELISA) |            |
| **Spores**          | Culture counting | [18, 46] |
|                     | Immunological detection |            |
|                     | Nucleic-acid based assays |            |
|                     | Ligand-based (Aptamers and Peptides) detection |            |
|                     | Biosensors |            |
| **Toxins**          | Radioimmunoassay | [20, 47, 48] |
|                     | Enzyme-linked immunosorbent assay |            |
|                     | Lateral flow |            |
|                     | Electrochemiluminescence (ECL) |            |
|                     | Biosensors |            |
|                     | Fluorescence |            |
|                     | Förster resonance energy transfer (FRET) |            |
| **Parasites**       | Fluorescent microscopy | [25, 26] |
|                     | Flow cytometry |            |
|                     | Automated blood cell analysers |            |
|                     | Serology antibody detection |            |
|                     | Molecular methods |            |
|                     | Laser desorption mass spectrometry |            |
|                     | Enzyme-linked immunosorbent assays (ELISA) |            |
|                     | Indirect fluorescence antibody test (IFAT) |            |
interactions and the polymerase chain reaction (PCR) method which involves DNA analysis are being used. These methods can be sensitive, inexpensive and give qualitative and quantitative information, however, a pre-treatment of the samples is needed; furthermore they are greatly restricted by assay time [37]. The electrochemical detection methods possess several advantages such as easy operation, low cost, high sensitivity, simple instrument and suitability for portable devices. However, to improve their performance one of the most popular ways is to use nanomaterials with a high surface area to functionalize the electrode [4, 5, 10]. In this context, nanomaterial-based methods have demonstrated improvement of the sensitivity, but due to reproducibility problems, as well as interferences, their application in real samples is still limited.

3.2.1 Bacteria and Spores

The prospect of nanomaterials is promising for rapid and sensitive pathogen detection [12, 14–16]. Current literature shows numerous applications of different nanostructures in biosensor devices for the detection of pathogenic microorganisms that are of importance to food and environmental safety, biosecurity, and medical diagnostics.

In this section, some aspects related to the detection of Salmonella, E. Coli, B. anthracis and Clostridium botulinum by using metal nanoparticles such as Au NPs, Cu@Au NPs, MnPs and QDs will be described.

Nanotechnology gives new approaches in order to detect microorganisms through the use of nanomaterials. This field has been explored by Lin et al. [12] using screen-printed carbon electrodes (SPCEs) modified with Au NPs (13-nm diameter) and ferrocenedicarboxylic acid (FeDC). The detection method consists of a sensitive detection of horseradish peroxidise (HRP) activity coupled with Au NPs and FeDC to amplify the amperometric effect. This has the potential for further applications in the rapid pathogen detection. One important advantage of these amperometric immunosensing strips is that approximately 50 CFU of E. coli O157:H7 in milk samples can be detected in 1 h. In this same context, Zhang and co-workers [13] described a sensitive electrochemical immunoassay for rapid detection of E. coli by ASV based on core–shell Cu@Au nanoparticles (NPs) as anti-E. coli antibody labels.

M NPs due to their high surface/volume ratio offer more contact surface area for attaching carbohydrates and for capturing pathogens. Based on this, E. coli detection using epifluorescent microscopy has been performed by functionalizing the surface of M NPs with D-manosse sugar (man-M NPs) through an amide linkage [14], subsequently incubations with fluorescein-labeled concanavalin A (Con A) and E. coli cells in phosphate buffer solution (PBS). After that, a magnetic field was applied for separating M NPs/E. coli aggregates (see Fig. 3.1a). The supernatants were removed and the remaining aggregates were washed thoroughly, stained with a fluorescent dye (PicoGreen), transferred to a glass slide, and imaged. Fluorescent
Microscopic imaging showed that *E. coli* can be detected (see Fig. 3.1b) with a limit of detection $10^4$ cells/mL by obtaining a high capture of bacteria.

Metal nanoshells based nanomaterials that exhibit a surface plasmon resonance (SPR) are also used for *E. coli* detection. Metal nanoshells are thin coatings (a few tens of nanometers thick) on large particles (a few hundreds of nanometers in diameter) which form the cores. Researchers have demonstrated a rapid and reliable test for the detection of *E. coli*, by using the SPR band associated with the coupling of the antibodies to the silver nanoshells [15]. This detection method has shown that the *E. coli* antibody interaction is extremely specific and that the presence of other microorganisms could not produce changes in the SPR band. Furthermore, it could help to shorten the testing time of drinking water used as sample with interest in possible terrorist attacks beside other applications related to the human health.

Electrochemical immunoassays based on Au NPs have also attracted considerable interest for *Salmonella* determination due to its simplicity, high sensitivity, inexpensive instrumentation, and miniaturization. For example, a highly sensitive strategy based on Au NPs for detecting *Salmonella typhi* (*S. typhi*) has been studied by Dungchaia et al. [17]. They immobilized monoclonal antibodies (McAbs) on polystyrene microwells and captured *S. typhi* bacteria by using a copper-enhanced Au NPs label coupled with anodic stripping voltammetry (ASV). The amount of

\[ \text{Fig. 3.1 E. coli detection using epi-fluorescent microscopy. (a) Schematic representation based on the functionalization of the silica coated magnetite nanoparticles, (b) (above) Increase in the fluorescent emission spectra for increasing concentrations (cells/mL) of E. coli., (below) TEM images of MGNP 3/E. coli complexes (Adapted from [14] with permission)} \]
deposited copper was related to the amount of Au NP tag present, which was controlled by the amount of S. typhi attached to the polyclonal antibody–colloidal gold conjugate. Therefore, the anodic stripping peak current was linearly dependent on the S. typhi concentration with a detection limit as low as 98.9 CFU/mL with interest for real samples analysis with a low detection limit, high accuracy, and fast analysis time.

Au NPs modified with Salmonella spp McAbs can also be evaluated using the electrochemical impedance spectroscopy (EIS) technique as an efficient method for fabricating a capacitive immunosensor for the detection of Salmonella spp. in real samples [16].

B. anthracis is another of the biggest threats special because of its potential use in bioterrorism. B. anthracis spores can be transmitted easily to humans. These spores are highly resistant to normally destructive environmental factors to living cells, such as heat, toxic chemicals, desiccation, and physical damages. These properties make them suitable for a potential biological warfare [49]. For this reason, the rapid and accurate detection of B. anthracis spores in the environment prior to infection is very important for human safety and national security. However, few technologies have been widely evaluated under field conditions [46].

Accordingly, nanomaterial-based biosensors are evolving as promising alternatives to meet this challenge in terms of sensitivity, specificity, time- and cost-efficiency [30]. For this, label-free (bio)sensing systems based on nanomaterials present certain advantages in detection. Among label-free assays, the quartz crystal microbalance (QCM) piezoelectric sensor has proved to be a useful platform in the efficient detection of pathogens including B. anthracis. Based on this platform, electrically active magnetic (EAM) nanoparticles are being used as concentrator of DNA targets as well as electrochemical transducers for detection of the B. anthracis protective antigen A (pag A) gene [18]. More details can be seen in the Fig. 3.2.

Another biosensor based on QCM has been developed by R. Hao and coworkers for B. anthracis spores detection by an anti-B. anthracis monoclonal antibody designated to 8 G3 (mAb 8 G3, IgG) functionalized QCM sensor [50].

### 3.2.2 Toxins

Neurotoxins produced by Clostridium botulinum, serotypes A through G (BoNT A-G), are considered the most potent toxins known to humans who cause neuro-paralytic disease [51]. The “Class A agents” are listed as one of the six highest risk threat agents for bioterrorism [22]. Food-borne botulism is the most common intoxication form due to the ingestion of pre-formed Botulimum Neurotoxin (BoNT) in food. For this reason, the development of nanomaterial-based rapid methods that can help to detect terrorist agents, such as anthrax, BoNT, etc. is very important for safety and security purposes.

Colloidal gold has emerged as the preferred label [52] for toxin detection. Alternative rapid methods, as colloidal gold-based immunochromatographic assays
(ICA) (also called lateral flow (LF) or strip assay) have been developed for detection of botulinum neurotoxin type B (BoNT/B). This class of assays was based on the sandwich format using polyclonal antibody (Pab). A thiophilic gel purified anti-BoNT/B Pab was immobilized in a defined detection zone on a porous nitrocellulose membrane and conjugated to colloidal gold particles as a detection reagent. The BoNT/B-containing sample was added to the membrane to react with Pab-coated particles, thus, a change of colour was given in the detection zone with an intensity of red colour proportional to BoNT/B concentration.

The strip assay exhibits potential as a rapid and simple assay method for the detection of toxin in biological fluid that requires no separation steps. Moreover, in function of these characteristics the assay can be considered superior to other immunoassay, such as radioimmunoassay and enzyme-linked immunosorbent assay [20].

Another test based on ICA has been reported by Klewitz et al. [21] to detect botulism neurotoxin D (BoNT/D). The test was based on double sandwich format using a gold-anti BoNT/D monoclonal antibody conjugates (detector reagent) and an anti-BoNT/D chicken polyclonal antibody. Feacal samples or standard samples spiked with various concentrations of BoNT/D were treated in gelatine-phosphate buffer, vortexed and stored at temperature ranging 5–8°C overnight. Polyclonal chicken anti-BoNT/D IgG and gold conjugated monoclonal antibody were added to the sample extracts and incubated at 37°C for 3 h. Then, 50 µL of pre-treated

![Fig. 3.2 Electrically active magnetic (EAM) nanoparticles in B. anthracis DNA detection.](image-url)

(a) TEM image of EAM nanoparticles, (b) Schematic representation of the EAM based electrochemical DNA biosensor detection principle, (c) Electrochemical response of the biosensor to different target DNA concentrations (mean current ±SD, n = 3) (Adapted from [18] with permission)
samples were applied to the test strip. The intensity of colour, of the red test line (signal intensity), is directly related with the concentration of BoNT/D in the standard or spiked horse faecal samples. Thus, toxin concentrations were determined within 3.5 h down to 50 pg/mL.

In the last years, several researchers have used luminescence methods instead of colorimetric methods for (bio)sensing applications. A novel class of luminescence nanomaterial is emerging for botulinum toxin assays [22]. This is the case of colloidal semiconductor QDs, which are single crystals of few nanometers of diameter whose size and shape can be precisely controlled by the duration, temperature, and ligand molecules used in their synthesis [53]. These nanomaterials have unique optical properties such as high extinction coefficients over a wide wavelength range, size dependent optical emission (due to quantum confinement effects on the electronic structure of the QDs) and relatively high quantum yields, which makes them suitable for biological applications such as fluorescence immunoassays, DNA array technology, fluorescence labeling of cells and tissues, and in the detection of chemical and biological agents. In Fig. 3.3 is shown an example of a fluorescence sandwich immunoassay using high-affinity antibodies and quantum dot (QD) reporters for detection of BoNT serotype A (BoNT/A) using a nontoxic recombinant fragment of

Fig. 3.3 Detection of BoNT/A-HC-fragment with bead-based immunoassay. (a) Scheme of the sandwich immunoassay: AR4 primary antibody specific for BoNT attached to a solid support as Sepharose bead; RAZ1 secondary antibody attached to the reporter 655 nm quantum dot and bound to the toxin fragment-primary antibody complex, (b) Calibration curve for the detection of BoNT/A-HC-fragment in FBS and (c) Detection from BoNT/A-HC-fragment in buffer using 655 nm QDs and detection on a flow system (Adapted from [22] with permission)
the holotoxin (BoNT/A-H$_C$-fragment) as a structurally valid simulant for the full toxin molecule [22].

Recently, a detection method based on the use of semiconductor QD-peptide Förster resonance energy transfer (FRET) assemblies to monitor the activity of the BoNT serotype A light chain protease (LcA) has been reported by Sapsford et al. [48]. They evaluated the ability to self-assemble dye-labeled LcA peptide substrates by using a number of different QD materials displaying charged or PEGylated surface-coatings by monitoring FRET interactions. Furthermore, Grate and co-authors [47] have developed for botulinum toxin detection an electrochemiluminescence (ECL) method based on a sandwich complex with paramagnetic beads and capture of antibodies bound to the beads by a streptavidin-biotin linkage, and the detection of antibodies labeled with a ruthenium chelate.

Historically, nanoparticles have been used as label biomolecules. Their properties coupled with the ability to attach nearly any biologic recognition element to the particle surfaces, facilitates their application in multi-target assays. These nanomaterials can be conjugated to DNA sequences or proteins so that, using size-dependent properties as just one example, fluorescence or light scattering can be used as an output signal, respectively. For example, a method for analyzing combinatorial DNA arrays using oligonucleotide-modified gold nanoparticle probes and a conventional flatbed scanner has been studied by the Mirkin’s group [54]. Labeling oligonucleotide targets (based on the anthrax lethal factor sequence) with nanoparticles rather than fluorophore probes substantially alters the melting profiles of the targets from an array substrate. This difference permits the discrimination of an oligonucleotide sequence from targets with single nucleotide mismatches with a selectivity that is over three times that observed for fluorophore-labeled targets. In addition, when coupled with a signal amplification method in which silver ions are reduced by hydroquinone to silver metal at the surfaces of the Au NPs, the sensitivity of this scanometric array detection system exceeds that of the analogous fluorophore system by two orders of magnitude.

Labeling technology based on highly fluorescent europium (Eu$^+$) NPs could provide a rapid and sensitive testing platform for sensing bioterrorist agents. Lately a europium-nanoparticle based immunoassay (ENIA) for the sensitive detection of anthrax protective antigen has been reported [19]. The use of Eu$^+$ NPs further permits to the assay to be adapted to an ELISA format that is already in place in testing laboratories because the antibody-antigen sandwich complex bound to Eu$^+$ NPs coated with streptavidin (SA) can be directly measured with a fluorescence reader. This ultrasensitive NP-based assay for the detection of anthrax toxin could provide a useful new tool for infectious agents and chemical contaminants.

### 3.2.3 Viruses

The detection of infectious viral diseases is very important for the public health. In recent years, a number of viral outbreaks, such as severe acute respiratory syndrome (SARS), influenza A (H1N1 flu) and avian influenza A (H5N1 flu) are
emerging. These have raised significant fears due to that could rapidly spread and
turn into a pandemic similar to 1918 Spanish flu that killed more than 50 million
people [55]. For this, rapid and sensitive diagnostic techniques using different
nanomaterials are being developed for recognizing and controlling future epide-
mics. An example of this is the recent development of immunochromatographic strip
for rapid detection of H9 subtype avian influenza viruses (H9 AIVs) [23]. The
assay is based on a colloidal gold anti-hemagglutin monoclonal antibody conju-
gate (detection antibody) and an anti-Nucleocapside protein monoclonal antibody
used as a precipitation reagent on the test line of a nitrocellulose membrane. This
detection method is rapid and easy to operate without the requirement of special
skills and equipment, which makes it a strip suitable for field detection. In addition,
this generation of ICA allows doing multiple assays that can help to diagnostic of
common poultry diseases, such as Newcastle disease, avian infectious bronchitis,
avian infectious laryngotracheitis, etc. Another similar study has been reported by
the same authors to detect IgG antibodies against the nucleocapsid protein of AIV
subtypes (H5, H7 and H9) in chicken sera [56].

The use of immunosensors based on the identification of virus glycoproteins,
[57, 58] or genosensors based on the detection of specific DNA sequences correlated
to the virus RNA [59, 60], has been performed as an alternative to more expensive
classical methods that consume time. In Mexico and USA, in March–April 2009,
there was an outbreak of human H1N1 flu virus that created a pandemic concern
[61]. At the time more than 207 countries worldwide reported cases of pandemic
H1N1, including at least 8,768 deaths [62]. For this reason developing of accurate,
rapid and low cost sensing methods for the early detection of this kind of virus is of
great importance.

An impedimetric detection method of a DNA sequence correlated to H1N1 virus
using carbon nanotubes platform and Au NPs amplification could be considered a
good possibility for improving the sensitivity and rapidity of analysis [24]. Such
systems are based on the use of colloidal gold for the labelling of DNA oligonucle-
otides, and the electrochemical signal of Au NPs onto screen printed carbon nano-
tubes electrode is measured and correlated to the DNA target concentration.

3.2.4 Parasites

The use of parasites as bioterrorism agents has not received so much attention.
Parasites could contribute to the installation of fear in human population upon inten-
tional addition to their food and water supplies. In the last years, biosecurity issues are
gaining importance as a consequence of globalization. Surveillance is critical in main-
taining biosecurity and early detection of infectious disease agents is essential [63].

Infectious diseases, like malaria, are being one of the greatest health challenges
worldwide. Nanotechnology is one of the promising strategies for malaria treat-
ment. The identification of new Plasmodium or infected cell targets can be used to
modify existing drug delivery systems employing nanotechnology to more efficiently
deliver antimalarial drug molecules to the newly-targeted sites of action. Electrochemical immunosensors offer several advantages compared to alternative detection methods, including the ability to analyze the direct blood samples, high sensitivity, require low sample volume and can be used in field application [64]. For example, nanosized carriers are receiving special attention with the goal of minimizing the side effects of drug therapy, such as poor bioavailability and the selectivity of drugs. Several nanosized delivery systems have already proved their effectiveness in animal models for the treatment and prophylaxis of malaria [34].

A number of strategies for the detection of *Plasmodium falciparum* are being developed by using rapid diagnostic tests based on nanomaterials. Magnetoimmunoassay-based strategies for the detection of Plasmodium falciparum histidine-rich protein 2 (HRP2) related to malaria have been described by using M NPs [25]. The immunological reaction for the protein HRP2 was performed in a sandwich assay on M NPs by using a second monoclonal antibody labeled with horseradish peroxidase (HRP) enzyme. The modified M NPs were captured on the graphite-epoxy composite electrode surface using a magnet inside of the composite electrode which was used as transducer in the electrochemical detection. This magnetoimmunoassay based on magnetic nanoparticles has shown a limit of detection (LOD) of 0.36 ng mL$^{-1}$, which makes it a suitable method for *Plasmodium falciparum* histidine-rich Protein 2 detection related to malaria.

Recently, electrochemical immunosensors based on screen printed electrodes are (SPEs) attracting great interest [65] due to ease of fabrication, ability to mass produce, disposability and portability. Sharma et al. [26] have developed an amperometric immunosensor based on Au NPs/alumina sol–gel modified SPEs for antibodies to *Plasmodium falciparum* histidine rich protein-2 (*Pf*HRP-2) by dripping Al$_2$O$_3$ sol–gel on SPE followed by electrochemical deposition of Au NPs. The antibodies in rabbit serum sample were allowed to react with the *Pf*HRP-2 protein which was immobilized on the modified SPE to form antigen-antibody immune complex (*Pf*HRP-2/anti-*Pf*HRP-2). The bound antibodies were quantified by alkaline phosphatase (AP) enzyme labeled secondary antibodies (anti-rabbit immunoglobulins-AP conjugate). Enzymatic substrate, 1-naphthyl phosphate was converted to 1-naphthol by AP and an electroactive product was quantified using amperometric technique. Figure 3.4 shows some results of this electrochemical detection.

Although the electrochemical immunosensors together with the nanotechnology are providing certain advantages, in terms of improving the selectivity and sensitivity of the detection systems in the field applied to parasites detection, it is a domain that is still under development.

### 3.2.5 Explosives

The conventional methods to detect explosives are restricted by disadvantages such as expensive instrument usage and time-consuming processes. Therefore, it is important to develop methods for trinitrotoluene (TNT) assay with simplicity,
sensitivity, rapidity and cost-efficiency. Lately, methods for TNT assay have been developed by using Au NPs and QDs. For example, Jiang and co-workers \cite{66} reported a simple and sensitive method for colorimetric visualization of TNT at picomolar levels based on color changes of Au NPs in the presence of TNT via the electron-donor–acceptor interaction between TNT and primary amines. New methods have been tested for the determination of TNT based on fluorescence quenching of QDs. Chen et al. \cite{27} have reported a sensitive method with high selectivity for TNT detection by using water-soluble L-cysteine-capped CdTe QDs as fluorescence probe. L-cysteine is used as stabilizer of QDs and as primary amine provider. Intermediate complexes between TNT and cysteine are formed, resulting in the fluorescence quenching of the QDs. This method can be adapted for the detection of TNT. The LOD was 1.1 nM and specificity detection was achieved. More details are observed in the Fig. 3.5.

Other methods based on fluorescence detection have been reported by Wang and co-authors where the fluorescence of oleic-acid-covered CdSe QDs

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**Fig. 3.4** Detection of *Plasmodium falciparum* histidine rich protein-2. (a) Schematic representation of the modification and immobilization of biomolecules on screen-printed electrode (SPEs), (b) Amperometric signals for rabbit-anti PfHRP-2 (1:20,000 dilution) on (a) Bare SPE, (b) Al₂O₃-sol–gel/SPE, and (c) AuNPs/Al₂O₃-sol–gel/SPE immunosensor in DEA buffer solution at pH 9.8, applied potential 400 mV vs. Ag/AgCl/sat.KCl reference electrode (Adapted from \cite{26} with permission)
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could be efficiently quenched by nitro aromatic analytes \cite{67}. Zhang et al. found that aminoligand-modified ZnS-Mn\(^{2+}\) nanocrystals can be introduced to detect TNT \cite{68}.

Recently, graphene owing to its unique properties, such as remarkable electronic conductivity, incredibly large electroactive surface area, high affinity and electrocatalytic activity \cite{69} is considered another of the interesting nanomaterials to be used for nitroaromatic explosives detection. Guo et al. \cite{28} have evaluated a new ionic liquid (IL)-graphene composite prepared by combining IL and graphene with large specific surface area and pronounced mesoporosity for ultratrace explosive trinitrotoluene detection. On the other hand, the determination of 2,4-Dinitrotoluene (2,4-DNT) by means of electrochemically reduced graphene on glassy carbon electrode (GCE) has been analyzed by using electrochemical detection, which gave a low detection limit of 42 nmol L\(^{-1}\) (S/N=3) \cite{29}.

![Fig. 3.5 L-cysteine-capped CdTe QD-based sensor for trinitrotoluene (TNT) detection. (a) HRTEM image of CdTe QDs, (b) Schematic representation for the L-cysteine-capped CdTe QD-based sensor for TNT detection, (c) FL spectra and the quenching efficiency (inset) of L-cysteine-capped CdTe QDs in the presence of different concentrations of TNT (from (a) to (i) 1.1 \times 10^{-9}, 5.5 \times 10^{-9}, 2.2 \times 10^{-8}, 8.8 \times 10^{-8}, 2.2 \times 10^{-7}, 8.8 \times 10^{-7}, 4.4 \times 10^{-6} and 1.1 \times 10^{-5} M) (Adapted from \cite{27} with permission)
3.3 Conclusions

Nanotechnology could provide unlimited opportunities for improving the efficacy of (bio)sensing systems for bioterrorist agents. In this chapter are presented some of the detection methods more commonly used for bioterrorist agents as well as for other safety and security applications. However, the domain that nanotechnology has in the detection of bacteria, toxins, parasites, viruses and explosives is still in development phase. To overcome the challenges of nanomaterial-based (bio)sensing strategies for safety and security applications a more detailed study related to interferences for real sample analysis as well as technological aspects related to final application need to be addressed.

Point strategies to overcome the challenges should be (a) In-field applications. In-field applications of nano-biosensing still need a big effort so as to overcome problems related to applications in real samples. Avoidance of interferences coming from sample matrix is the key point for success; (b) Low detection limits. Reaching of low detection limits (detection of few molecules, bacteria, cells, etc.) in a relatively high volume of samples (i.e. 1 molecule or 1 cell in 1 mL food sample) needs the development of fast and efficient preconcentration tools/routes based on nano & microfabrication.

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