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# Biosensors for the Detection of Plant and Animal Diseases: Recent Advances and Future Perspectives

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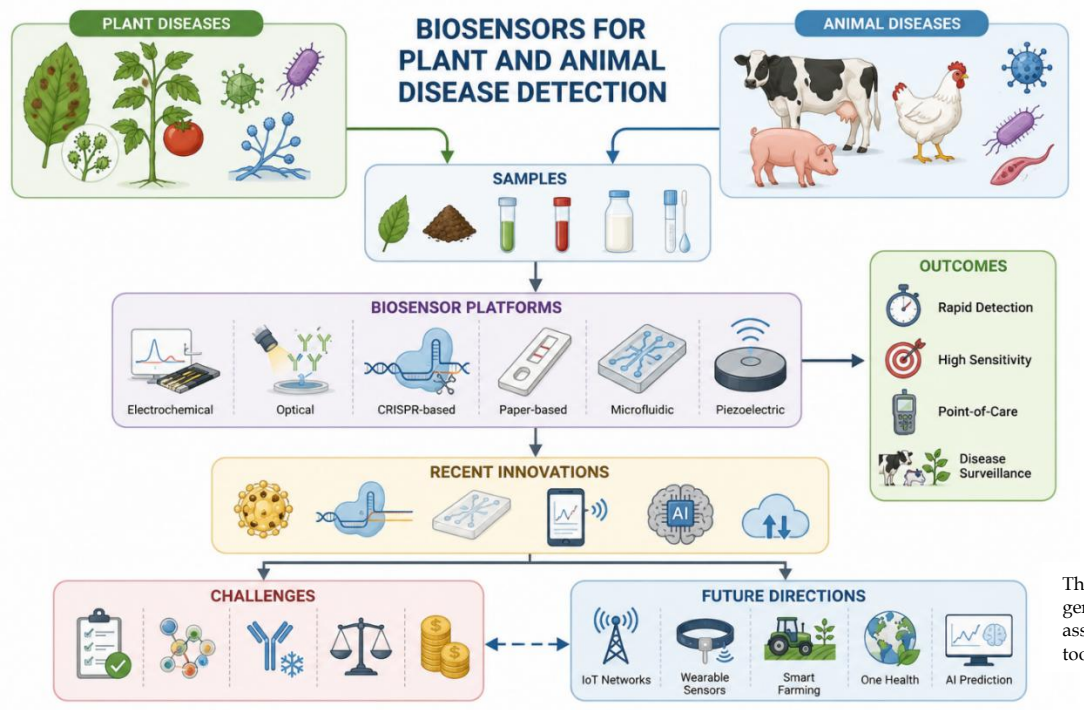
## Abstract

Diseases affecting plants and animals have a huge cost globally and threaten food security and economies. Pests and pathogens account for almost forty percent (≈$220 billion) of global crop losses each year, and livestock diseases cost (e.g. $358 billion per year) hundreds of billions of dollars globally. Culture, ELISA and PCR are reliable, but time-consuming, laboratory-based, and costly. In this context, biosensors, devices that integrate the biological recognition element, the bioreceptor, with a physical transducer, have emerged as a game-changer in terms of fast and sensitive detection of disease and field deployable applications. New developments such as the use of nanomaterials, CRISPR-Cas, microfluidics and AI have enhanced the portability and performance of the biosensor. However, there are still some difficulties in reagent stability, validation, cost and regulation. The review outlines the principles of biosensor design, includes representative examples of plant and animal pathogens and discusses the various metrics of different biosensor platforms, and outlines some case studies of commercialization. The article highlights the innovations in precision diagnostics of disease that have occurred over the past few years and introduces some of the trends expected to appear in the future. The overall innovations of biosensors can alter the disease management course in the general terms. By allowing for early detection of an outbreak on site, which can stop an outbreak or help improve yield or One Health surveillance.

**Graphical Abstract/Summary**:




This graphical abstract was generated using an AI-assisted image generation tool.

**Keywords:** Biosensor, Point-of-care diagnostics, Veterinary diagnostics, Plant pathogens, CRISPR diagnostics, Field-deployable biosensors, Nanotechnology

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## Introduction

Virus, bacteria, fungi and parasites that attack plants and animals are the enemies of global health and agriculture. Plant diseases limit yield and quality of crop [1]. Famine-causing rust fungi or *Phytophthora infestans* (late blight) have been the cause of billions of dollars of damage over the years [2]. Pathogens with broad spectrum have made more threat to modern agriculture with its extended plant densities and climate change. Losses due to pests and diseases are estimated to be as high as 40% of the crops per annum. This is estimated to be at a cost of approximately $220 billion annually [3]. Outbreaks in animals such as foot and mouth disease, swine fever, avian influenza and brucellosis can cause significant economic losses because of the need to slaughter animals. Every year, livestock diseases cause the loss of 358 billion dollars worth of production globally. So, timely detection and treatment is crucial [4].

Although they are limited, traditional tests are still considered to be the benchmark in agriculture for disease diagnosis [5]. Laboratory-based tests need expensive infrastructure; require a lot of time to run (often hours to days) and do not have the flexibility to be applied in situ [6]. For example, PCR and qPCR are very sensitive, and they take 2-4 hours and cost per test approximately $10-30. Time and cost eat up ability to respond quickly in the event of outbreaks. With this, if not treated, infections can spread quickly. Furthermore, a lot of farming communities do not possess sufficient labs and human resources [7].

This is where biosensors come in, providing on-site, real-time analysis. In brief, a biosensor is a device containing a biorecognition component (an antibody, enzyme, probe containing nucleic acid, or aptamer) which specifically recognizes the target pathogen (or a biomarker), and a transducer which generates a measurable response (electrical, optical, etc.) when the biorecognition component binds the target. The biosensors can give quick results at point-of-need by doing away with lengthy culture or multiple pipetting steps (minutes-scale) [8]. There have been several reviews covering specific biosensing platforms such as plant pathogen biosensors, veterinary diagnostics, point-of-care devices, nano-biosensors and CRISPR-based diagnostics. Most of these focus on a specific application or sensing approach. Plant health and animal health are not as extensively compared in the literature as are other types of biosensors. Furthermore, they have not been assessed by experts with respect to their analytical performance, commercialization, field validation, regulatory status and their future incorporation in one health surveillance systems. The review will try to overcome these shortcomings by comparing different platforms of traditional and emerging biosensors, such as nanotechnology-based, microfluidic, AI-based, smartphone-based, CRISPR/Cas-based, etc. It also identifies the difference between technologies available in a commercial product and those that are still a laboratory prototype. Additionally, it talks about the primary obstacles which hinder the adaptation of lab-based studies to the field [9], [10].

## Literature Search Strategy

A systematic literature search was conducted to uncover relevant records on biosensor technologies in animal and plant disease detection. Electronic databases such as PubMed, Scopus, Web of Science, ScienceDirect, IEEE Xplore and Google Scholar were used for searches. All articles dated from 2022 to 2026 were considered. Studies that were published prior to 2022 and were scientifically relevant were also included if they were considered landmarks. The search terms encompassed biosensors, plant diseases detection, animal diseases diagnosis, electrochemical biosensors, optical biosensors, paper based biosensors, microfluidics, lab-on-a-chip, CRISPR diagnostics, nano-biosensors, point-of-care diagnostics, and veterinary diagnostics. Peer-reviewed articles in English language were considered. These publications/Articles are not included in the review: Conference abstracts, Duplicate publications, Editorials, Studies lacking methodological details. Original research articles were preferentially selected for a report on analytical performance including limit of detection, sensitivity, specificity, assay time and field validation. The review article was used mainly to present background information on the topics in hand, and to summarize the technological developments at a high level.

## Biosensor Fundamentals

A biosensor usually consists of a biorecognition layer (bioreceptor), transducer, and electronic signal processor/display. The antibody, enzyme, aptamer(nucleic acid), nucleic acid probe, and even the entire cell or organism can be used as a bioreceptor for the recognition of the desired target like antigen, nucleotide sequence, toxin. This bioreceptor is very attached to the surface of the sensor [11]. When the target analyte binds to the transducer layer, it is converted into a measurable physical signal such as the change in electrical current/voltage (electrochemical), the change

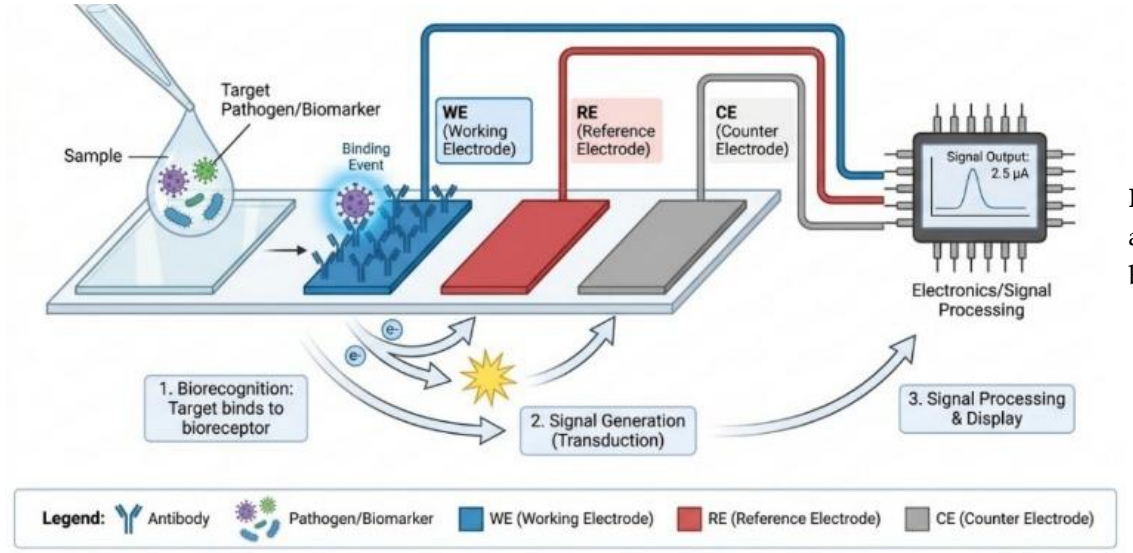



**Figure 1:** Working of an electrochemical biosensor.

in light intensity or color (optical), the change in frequency (piezoelectric) or the thermal change. Lastly, the electronic system amplifies and processes this signal to be read out [12], [13].

Biosensors are classified based on the type of bioreceptor and the method of transduction. Common classifications include electrochemical biosensors, optical biosensors, piezoelectric (mass-sensitive) biosensors, thermal biosensors, magnetic or mechanical sensors, paper-based biosensors [14].

The electrochemical biosensors quantify electrical changes, current or potential, caused by redox reactions or charge changes. Usually, they employ enzyme catalysis or immune sensing on electrodes [15]. The optical biosensors respond to changes in light absorbance, fluorescence, surface plasmon resonance, etc., due to biorecognition [16]. Mass-sensing devices (mass-sensitive biosensors) such as quartz crystal microbalances or cantilevers are used to measure minute mass changes (e.g. antibody-antigen binding) by resonant frequency change [17]. Thermal biosensors provide data on changes in temperature that occur during biochemical reactions (not common in field biosensors) [18]. While the magnetic or mechanical sensors are newer types that use magnetic beads or mechanical deformations [19]. Paper-based biosensors are frequently used colored nanoparticles or enzymes on paper strips (lateral flow assays) used to provide a simple visual readout [20].

The general electrochemical biosensor design is shown in **Figure 1**, where the pathogen analyte reacts with the bioreceptor layer (such as antibodies or DNA probes) which is selectively attached to the working electrode. The working electrode (WE) is functionalized with a bioreceptor (e.g. antibody or enzyme) that binds the target pathogen or biomarker in the sample. The transducer converts the binding event into an electrical signal (current, voltage, or impedance) measured against a reference electrode (RE) and counter electrode (CE). This signal is processed and displayed by the electronic system [15].

Target specificity is selected for biorecognition elements. Common bioreceptors include antibodies/immunosensors, nucleic acids, and whole cells or peptides. Antibodies/Immunosensors provide strong binding to particular proteins or pathogens. It is widely used in ELISA-type and lateral flow assays. Catalytic amplification (e.g. glucose oxidase) is another bioreceptor, that may be sensitive to conditions. Nucleic acids (DNA/RNA probes or aptamers) are designed to bind to a specific sequence (pathogen genome) or small molecules while the aptamers are synthetic alternative to antibodies. The whole cells or peptides can provide broad recognition or metabolic responses (more common in environmental sensors) [21], [22].

Electrodes (carbon or gold), optical fibers or chips, piezoelectric crystals and even smartphones (with cameras as detectors) are all examples of transducer elements. This combination determines the performance of the sensor [23]. Electrochemical sensors are very sensitive, miniaturizable, while optical sensors such as Surface Plasmon Resonance (SPR) or Fluorescence can be very specific but might be more complex with the optics [21]. Paper-based assays such as pregnancy tests are very inexpensive and easy to use, but less sensitive. To summarize, the design of a biosensor involves compromising the sensitivity, specificity, rate, cost and field practicability. Novel transducer surfaces with large surface area and conductance (material advances such as nanomaterials, conducting polymers, 2D materials) have resulted in improved signal and stability. The innovations mentioned below for the diagnosis of plant

and animal diseases are based on these fundamentals [24], [25].

## Biosensors For Plant Disease Detection

There are a number of pathogens that affect plants that include fungi, bacteria, viruses, nematodes and oomycetes. These include important fungal pathogens such as *Puccinia* (rusts), *Magnaporthe oryzae* (rice blast) and *Phytophthora infestans* (late blight pathogen of potato and tomato) [26]. *Xanthomonas spp.* is a bacterium that causes disease. Wilts, blights and cankers are caused by *Pseudomonas syringae* [27]. The most frequent viruses infecting plants include the *Tobacco Mosaic Virus, Tomato Yellow Leaf Curl Virus* and *Potato virus Y*. These viruses are readily spread from plant to plant and lack a good chemical control [28]. Nematodes (root-knot Meloidogyne spp.) and oomycetes also pose a threat to the roots and seedlings. Plant diseases annually result in losses of billions of dollars that impact on food security [29]. Figure 2 summarizes the infection biomarkers targeted by plants.

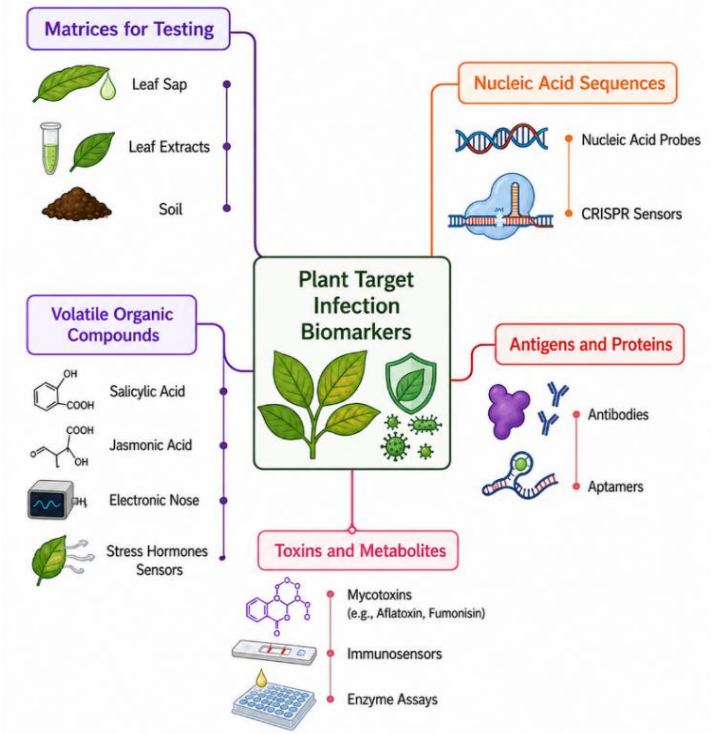



**Figure 2:** Major biomarkers targeted by biosensors for plant disease detection.

The introduction of nanomaterials and advanced molecular technologies has been widely adopted in the development of biosensors for plants [30]. Bacterial wilt pathogens have been targeted with the development of electrochemical immunosensors. The immunosensor which was developed consisted of a SPCE (screen-printed carbon electrode) that was modified with anti-*Xanthomonas oryzae* antibody. This SPCE was able to detect *Xanthomonas oryzae* down to $10^2$ CFU/ml oryzae variety bacterial blight of rice [31], [32], [33]. This electrode detected current changes as a result of antibodies binding to bacteria from rice extracts. Similarly, optical sensors were aimed at the coat proteins of viruses [32]. Also, scientists developed an electrochemical immunosensor based on the label-free AuNP/graphene composite to detect *beet necrotic yellow vein virus* at femtogram level [34].

Another type of biosensor frequently used is a nucleic-acid biosensor. For this reason, a LAMP (Loop-Mediated Isothermal Amplification) based CRISPR lateral-flow assay was used to detect viral RNA with high specificity, which will be a promising technology for a variety of applications [35]. Fungal pathogens have been detected using a nanoparticle-enhanced colorimetric assay, for example researchers developed $MoS_2$–Au nanocomposite sensors for the detection of the pathogen associated with sugarcane white leaf disease at sub-picomolar levels [36]. Also, the use of alternative biomass markers has gained attention. For instance, volatile organic compound (VOC) sensors installed into a smartphone were used to detect late blight. A team of scientists developed a VOC sensor array that was placed inside a smartphone and could detect *Phytophthora infestans* after two days of inoculation (95% accuracy). The VOC sensing can be scaled up to field-ready devices [37], [38].

To sum up, there is a variety of different plant biosensors. The assays for specific pathogens such as *Xanthomonas*, for example, or *Potato virus Y, Agrobacterium*, are rapid and are typically combined on printed immunosensors (electrochemical and optical), respectively [34], [39]. The CRISPR tools based on DNA/RNA show the ability to detect ultra-sensitive viral/bacterial genomes [40]. Portable examples consist of lateral-flow kits for *Phytophthora* and other targets as well as smartphone readers for colorimetric strips. These sensors can detect the disease at an early stage; often before symptoms appear, which is critical [41].

## Animal Disease Detection by Biosensors

There are also a number of infectious agents in the environment which can affect domestic animals such as livestock and pets [48]. Domestic animals such as dogs, cats, birds, as well as livestock animals such as cattle, buffaloes, pigs, sheep, goats etc. are zoonotic threats. Rapid assessment is key to outbreak management and zoonotic spillover control [49], [50].

**Table 1.** Comparison of Biosensor Technologies for Plant Disease Detection

| Technology | Target Pathogen/Biomarker | Sample | Typical LOD | Assay Time | Performance | Development Status | Key Advantages / Limitations | References |
|---|---|---|---|---|---|---|---|---|
| Electrochemical | Bacteria, viruses | Leaf sap | $10^2$–$10^3$ CFU/mL | 10–30 min | High | Prototype/ Field-tested | Rapid, portable; electrode fouling | [42] |
| Optical | Pathogens, toxins | Leaf tissue | pg–ng/mL | 15–40 min | High | Laboratory | Label-free; costly instruments | [43] |
| Paper-based (LFA) | Plant viruses | Leaf sap | $10^3$–$10^4$ copies | 10–20 min | Moderate–High | Commercial/ Field-tested | Low cost; lower sensitivity | [44] |
| Microfluidic (LOC) | DNA/RNA | Plant extract | 10–100 copies/µL | 20–45 min | Very High | Prototype | Automated; fabrication complexity | [45] |
| CRISPR/Cas | Viral/Bacterial DNA | Leaf extract | 1–100 copies/µL | 20–60 min | Very High | Emerging | Ultra-sensitive; nucleic acid extraction required | [46] |
| Nano biosensors | Proteins/Nucleic acids | Plant tissue | pg/mL | 10–30 min | Very High | Laboratory | Signal amplification; reproducibility issues | [47] |

**Abbreviations:** LOD, limit of detection; CFU, colony-forming units; LFA, lateral flow assay; LOC, lab-on-a-chip; pg, picogram; ng, nanogram; µL, microliter

Certain animal biosensors are able to detect certain biomarkers (a viral antigen or fragment of the genome, an antigen of bacteria or parasites, or antibodies generated by the host as a response to the virus). Usual sample types are blood/serum, milk, saliva, nasal swabs, feces or exhaled breath/sweat [51], [52]. For example, the clinical signs of foot and mouth disease may be observed in milk or saliva. The proteins that are measured in the sensor that are relevant for zoo-sanitary monitoring may either be proteins of the pathogens (antigens) or proteins of the host (antibodies, acute phase proteins) [53], [54].

On-farm diagnostics for livestock health management is now possible with biosensors. A new review indicates that nano-luciferase biosensors have been applied in the detection of *African Swine Fever Virus* (ASFV) antibody. The development of the split luciferase (LgBiT/SmBiT) was used as the basis for the development of a nano-BiT luciferase immunosensor for ASFV IgG. An ASFV viral antigen was fused to the split luciferase. Binding of serum antibodies induced luminescence. The assay was a 16-fold more sensitive than ELISA, and it was performed in a matter of minutes [55], [56].

Other livestock applications are immunosensors for *Brucella* in cattle, *Toxoplasma gondii* in sheep and *Mycoplasma* in pigs. Biosensors have also been developed for avian diseases. For instance, a portable, fluorescent assay for Newcastle disease virus [10]. Researchers also reported field-deployable biosensors for *Foot and Mouth Disease Virus* (FMDV) and *Avian influenza virus* (AIV) are employed in farms with a lateral-flow based sensor or an impedance sensor [57]. Beyond pathogens, wearable biosensors for animal health are emerging. Ear tags or collars with temperature, heart-rate or pH sensors can send out signals indicating fever or metabolic stress, which could be indicators of disease, before it becomes apparent. Sweat or breath sensors are also being studied as a way of tracking stress and infection [58].

In summary, the diagnostic devices that can be used either in the field or in the laboratory can be divided into many types. Electrochemical and optical immunosensors for detection of the specific pathogen, nucleic acid detectors for the detection of a virus, such as the CRISPR–based detection assays for foot-and-mouth disease, and integrated lab-on-chip devices with rapid PCR capability [59]. Researchers designed field PCR lab (mobile van) equipped with portable sequencer for on-site genotyping *African swine fever virus* (ASFV). The primary goals are as in plants, fast (<1-2h) and inexpensive point of care tests that are used in conjunction or in place of lab tests [60]. Recent research has shown that biosensors can have increased sensitivity and specificity to animal pathogens for on-farm surveillance [61].

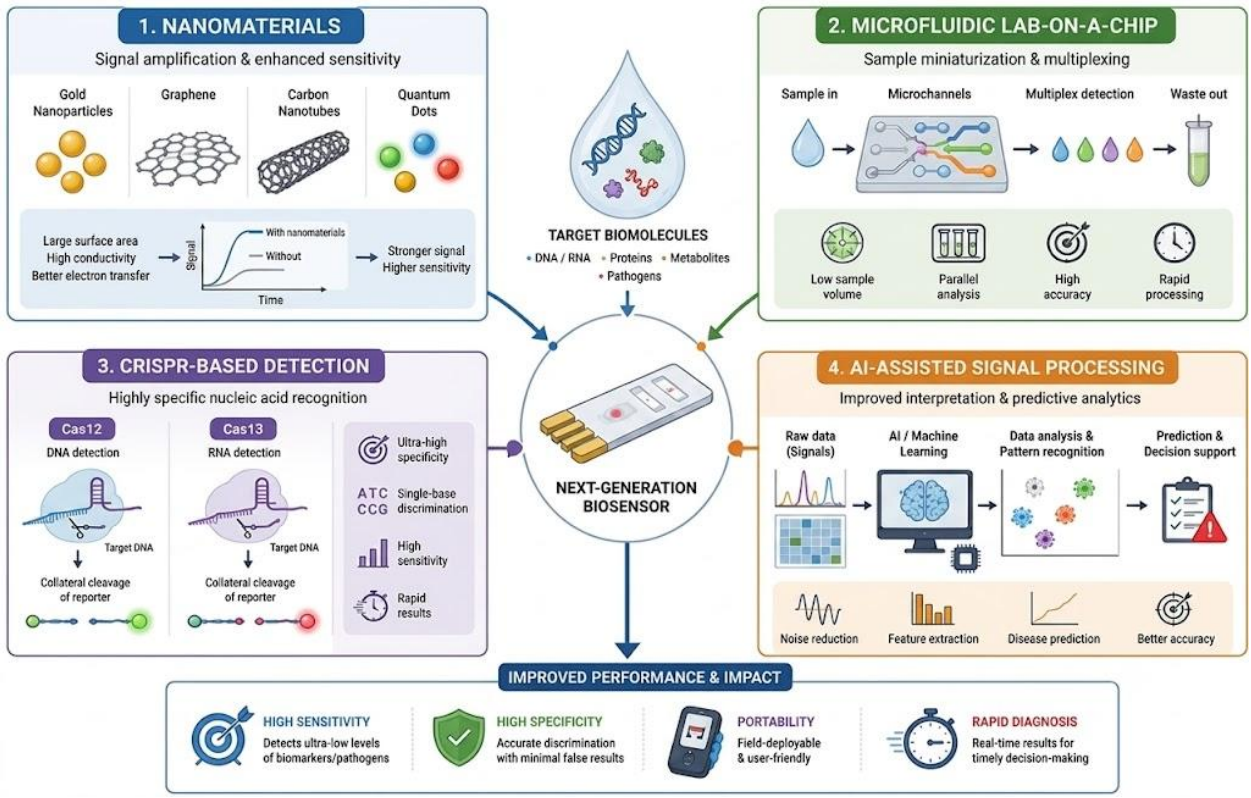



**Figure 3:** An overview of next-generation biosensors: Integrating nanomaterials, microfluidics, CRISPR, and AI to achieve ultra-sensitive, rapid, and portable detection of plant, animal, and human pathogens

Table 2. Comparison of Biosensor Technologies for Animal Disease Detection

| Technology | Target Disease/ Biomarker | Sample | Typical LOD | Assay Time | Performance | Development Status | Key Advantages / Limitations | References |
|---|---|---|---|---|---|---|---|---|
| Electrochemical | ASFV, FMDV, *Brucella* | Blood/Serum | $10^2$ copies/mL | 15–30 min | High | Prototype/ Field-tested | Rapid; matrix interference | [62] |
| Optical | AIV, BVDV | Serum/Swabs | pg/mL | 20–40 min | High | Laboratory | Sensitive; expensive | [16] |
| Paper-based (LFA) | ASFV, FMDV | Blood/Saliva | $10^3$ copies | 10–15 min | Moderate–High | Commercial | Simple; lower sensitivity | [20] |
| Microfluidic (LOC) | Viral DNA/RNA | Blood/Milk | 10–100 copies/µL | 20–45 min | Very High | Prototype | Multiplexing; complex fabrication | [63] |
| CRISPR/Cas | ASFV, FMDV, AIV | Blood/Swabs | 1–100 copies/µL | 20–60 min | Very High | Emerging | High specificity; cold-chain needs | [64] |
| Nano biosensors | Viral proteins | Serum | pg/mL | 10–30 min | Very High | Laboratory | Excellent sensitivity; scalability issues | [65] |

**Abbreviations:** LOD, limit of detection; SPR, surface plasmon resonance; LOC, lab-on-a-chip; ASFV, African swine fever virus; FMDV, foot-and-mouth disease virus; BVDV, bovine viral diarrhea virus; AIV, avian influenza virus

## Comparison of Plant vs Animal Biosensors

There are some common elements between the principles of biosensors used for plant and animals disease diagnostics, but there are distinct differences. Most plant diseases are diagnosed using sap, extracts from the leaves, soil samples and volatile organic compounds, and diagnostic testing on animals takes place using blood, serum, saliva, milk and feces [25], [37]. Besides this, animal disease biosensors are likely to have more regulations considering animal welfare and food safety concerns, and zoonotic disease surveillance. All of these differences affect sensor design, sample preparation procedures and validation requirements [66], [67].

## Recent Advances In Biosensing Technologies

Recent advances in biosensing technologies are being determined by nanomaterials, microfluidics, CRISPR-based detection, and AI-assisted analytics, providing ultra-sensitive, portable, and real-time diagnostics across medicine, agriculture, and environmental monitoring. These innovations are reshaping how diseases and pathogens are detected [25], [68], [69]. A summary of recent advances is shown in figure 3.

## Nanomaterial-Enhanced Sensors

Nanotechnology has revolutionized biosensor technology by improving analytical performance and enabling rapid, highly sensitive detection of target analytes. Incorporation of nanomaterials (nanoparticles, nanotubes, graphene) might provide a higher sensitivity, lower detection limits and enable novel signal transduction mechanisms. For example, gold nanoparticles (AuNPs), silver nanoparticles (AgNPs) and quantum dots possess strong plasmonic or fluorescent labels [70].

In sensors, nanomaterial modified (carbon nanotubes, graphene oxide, metal oxides) electrodes enhance surface area and conductivity. There are numerous plant pathogen detection sensors developed that are based on nanotechnology [71]. For example, chitosan coated iron-oxide NPs and Au-graphene oxide composites have a detection limit of 10-12 to 10-15 M, respectively. These types of sensors often enable label-free detection of pathogens or toxins at ultralow concentrations, and sometimes no amplification is necessary [72], [73]. The detection of plant stress volatiles and pesticides at a very high sensitivity is being carried out using nanozymes and MIPs (Molecularly Imprinted Polymers) on nanofibers [74]. In the case of livestock, nanomaterials enhance field assays, such as the use of magnetic beads coated with antibodies to accelerate sample preparation, and gold nanostar particles tagged with lateral flow strips that make low-abundance antigens more visible [75].

The advent of nanomaterials has led to the development of more portable and robust biosensors. The nanotechnology is used to improve the signal by means of tiny sensor chips of 1-2 $cm^2$ that are widely available and placed in handheld readers or attached to cell phones. In many recent works; these advances are described [76].

## CRISPR/Cas-Based Biosensors

An important development is the use of CRISPR–Cas system for diagnostics. With the help of RNA, the CRISPR-Cas12 and Cas13 enzymes cut specific pieces of DNA or RNA, producing amplified signals which are usually as a result of collateral cleavage of reporter molecules [77]. When isothermal amplification like LAMP or RPA (Recombinase Polymerase Amplification ) is coupled with this, ultra-sensitive portable nucleic acid tests can be produced. What is important is that these tests can be performed with little or no equipment and with PCR-like sensitivity (detection limits ranging from single-copy to approximately 100 copies per reaction), within 15-45 minutes and at a low cost [6].

A one-pot LAMP–Cas12 assay was able to detect *Fusarium* species in less than an hour [78]. Except for reporting the target DNA single molecule, a separate study reported the detection of plant viruses with a lateral-flow strip based on Cas12a [79]. These CRISPR diagnostics have equal or better sensitivity compared to qPCR, but do not need one [80]. A new review emphasizes the plant diagnostic applications of CRISPR tools as a new platform that provide quicker and more field-friendly alternatives to PCR/ELISA [81], [82].

Despite current issues like off-target effects, sample preparation etc., assays using CRISPR technology are becoming increasingly multiplexed [83]. Multiple pathogens can be simultaneously detected in a single cartridge with the orthogonal Cas enzymes. Companies Mammoth Biosciences and IDT (Integrated DNA Technologies) are commercializing the CRISPR kits [84], [85]. Of course, portable readouts such as lateral flow strips and battery-powered fluorometers and smartphones are frequently employed in the CRISPR tests. These can deliver the same results without the incidence of any laboratory infrastructure at only a few US dollars per test. CRISPR-based biosensors are a significant improvement in sensitivity and ease of use for diagnostics in plants and animals [64], [86].

## Microfluidics And Lab-On-Chip Systems

Microfluidic technologies have emerged as an important platform for next-generation biosensing applications. The Lab-on-a-Chip (LOC) device integrates the sample preparation, amplification, and detection phases in a single sealed cartridge or chip [87]. These systems help to minimize operating steps and contamination risks. Hand-driven devices like syringes or simple pumps enable field RPA or digital PCR in the absence of electricity [88]. Otherwise, LOC devices are based, for example, on electrochemical LAMP (a portable device operating at 39°C delivering results in 20 min or on fluorescence detection in a small cartridge [89].

These microfluidic platforms often are linked to cloud or smartphone software to read out the data [90]. For

example, the "mChip" employs smartphone fluorescence detection to detect Xylella fastidiosa (olive pathogen) in 30 min. Systems like PlexBio, OptoSelect multiplex LOC (lab on a chip) system can simultaneously detect multiple pathogens of tomato. Technology based on color coded beads are used [91]. A novel, paper-based microfluidic device (μPAD) was used to identify a pathogen responsible for citrus greening in field-based conditions, by taking advantage of the capillary action. The device is designed to be used with a liquid sample [92], [93].

## Paper-Based And Lateral Flow Assays

Lateral Flow immune-Assays (LFAs) are still very useful paper-based biosensors, especially for lateral flow. They are inexpensive, power independent and highly visible making them extremely popular for field diagnosis [94]. The last decade has seen a dramatic acceleration in LFA developments, thanks to the use of nanomaterials, such as gold or magnetic particles to generate a color contrast, and fluorescent nanoparticles to amplify the signal in front of a lateral flow readout, and even in front of a lateral flow detection of CRISPR [95]. An example of such a LFD (Lateral Flow Device) is one for the detection of *Xanthomonas campestris,* which will be a very sensitive and easy to use LFD [96].

In many cases, smartphones come with LFAs these days [97]. Agdia has created an ImmunoStrip for citrus greening that features a visible test line after an amplification step on the lateral flow strip, that can be read by a smart phone app [98]. This method has provided quick diagnosis in the US citrus groves. Literature generally concentrates on plant advances, while LFAs have become the basis for animal diagnostics – for example, IDEXX FMD 3ABC Ab test for FMD and Brucellosis [99]. Future innovative LFAs in development would utilize more than one type of nanoparticles or quantum dots, to detect different colors of analytes on the same strip [100].

In conclusion, paper-based biosensors are an established technology and evolving. They may not perform as well as some of the current assays used in the lab with a sensitivity of ~ng/mL to μg/mL for proteins, but there is some progress with nanoparticles, SERS (Surface-Enhanced Raman Scattering), CRISPR, etc. [45], [101]

## Smartphone And AI Integration

A growing number of common devices and analysis tools are being applied to create modern day biosensors [102]. Smartphones are just mobile detectors: the camera allows to capture colorimetric or fluorescent signals, the CPU performs artificial intelligence algorithms and connectivity communicates to the cloud [103]. For instance, immune strips, microfluidic chips and camera-based electrochemical imaging (spectral analysis of the color of the electrodes) have evolved smartphone reader [104].

AI/machine learning paired with diagnostics based on images are on the horizon. Models that use deep learning accurately classify images of leaves according to disease [105]. One such example is ViT-SmartAgri, a tool to determine whether it is late blight or mosaic on the tomato. The apps are able to detect fluorescent dots or LFA lines with higher precision than the human eye. Moreover, sensor data such as RFID (Radio-Frequency Identification) and environmental IoT (Internet of Things) data can be used as input to the ML (Machine Learning) models to detect a potential outbreak at an early stage [106]. But biosensors do not operate in isolation. With the merger of IoT and AI technology they become one of the nodes of a digital surveillance network. Wearable biosensors, including smart collars, ear tags, and environmental monitoring devices, are increasingly being investigated for continuous monitoring of animal physiological parameters and environmental conditions associated with disease risk [102], [107].

In conclusion, biosensor technology is already becoming a part of a connected biosensor ecosystem with the development of instrumentation and data science [108].

## Comparative Analysis of Biosensor Platforms

It should be noted that specificity is based on the biorecognition element; some modern sensors particularly those using nucleic acids or aptamers can reach analytical specificities approaching approximately 100% under optimized laboratory conditions [109], [110]. The electrochemical biosensors typically have high sensitivity and low detection limit but may be influenced by fouling of the electrode and environmental factors [15]. . The good specificity and multiplexing of optical biosensors is often accompanied with complex instrumentation. Hence, they are used only in low-resource areas. CRISPR associated diagnostics have become a more sensitive alternative method of PCR [94]. . Still, issues regarding guide RNA design, off-targets and sample preparation are still serious impediments to large-scale field deployment [111]. . Paper based assays are less sensitive than laboratory-based assays and are inexpensive, but simple to use [112]. So, you should make the choice of biosensor platform depending on the requirements of the diagnosis [113]. The major biosensor

platforms have been compared in terms of analytical and practical metrics as shown in the Table 1.

In many biosensor platforms, conflicting results of field performances have also been reported. While some studies illustrate laboratory-level sensitivity, others indicate a drop in accuracy under field conditions due to environmental variability, sample diversity, and matrix effects. The differences illustrate the need for standardized validation protocols and multi-site field evaluations before widespread implementation can be achieved [114], [115], [116].

## Commercialization And Case Studies

Multiple biosensor technologies have evolved from lab prototypes to delivery or commercialization [117]. For example, Agdia Immunostrip® and Pocket Diagnostic® offers diagnostics kits for field testing plant pathogens (primarily LAMP and lateral flow) worldwide. These commercially available kits are designed for the rapid detection of plant viruses, bacteria, and fungi from leaf sap or plant tissue using immunochromatographic lateral flow technology and have been extensively validated for routine field diagnosis [41]. Diagnostic companies have begun to offer kits for the field that can be used for livestock. Veterinary practitioners can purchase lateral flow assays for Foot-and-Mouth Disease and Brucella brucellosis, bovine viral diarrhea, and Johne's disease from manufacturers such as IDEXX Laboratories, BioNote (Anigen Rapid), and Svanova, subject to several peer-reviewed case studies. These commercial assays primarily use serum, milk, or whole blood samples and are based on immunoassay principles, with extensive validation in veterinary diagnostic laboratories and disease surveillance programs [118], [119].

Researchers and collaborators have created a portable fluorescence immunosensor for the detection of BVD. They also created an ear-tag rumen pH sensor for monitoring and evaluating the health of the cow. These technologies represent field-tested prototypes that have demonstrated promising performance under practical conditions but require further multicenter validation, regulatory approval, and large-scale manufacturing before routine commercialization [10], [120]. Some of the microfluidic case studies include an integrated chip for the *Xylella fastidiosa* in olives and a colorimetric µPAD for several mycotoxins in grains integrated with a smartphone. Similarly, these microfluidic platforms have shown successful performance in pilot field studies but remain prototype technologies requiring further multicenter validation and regulatory approval before commercialization [67], [121]. Several startups are looking into CRISPR diagnostics for applications in food safety including pathogen detection in meat, vegetables, etc., but none of these products have reached commercialization. Accordingly, most CRISPR/Cas-based biosensors remain laboratory proof-of-concept technologies, while many nano-biosensor and lab-on-a-chip platforms are still under development and require further optimization, manufacturing standardization, and field validation before commercial translation [122], [123].

Any large-scale deployment of something must be well validated [124]. Lateral flow devices have been field-tested for banana wilt caused by *Xanthomonas* and it was found that local technicians were able to reliably use a simple dipstick test in order to provide early warning [125]. These findings demonstrate that commercially available lateral flow technologies can be effectively implemented under field conditions when supported by appropriate user training and standardized operating procedures. The introduction of on-the-spot testing for foot and mouth disease (FMD) and *Bluetongue virus* (BT) to livestock is a development that has recently been introduced and it is now being applied to dairy cattle by mobile milk-testing labs in developing countries [126]. Similarly, field deployment of veterinary biosensors has highlighted the importance of robust validation across diverse environmental conditions and sample matrices to ensure consistent diagnostic performance. Collectively, these case studies emphasize that successful commercialization depends not only on analytical accuracy but also on comprehensive field validation, regulatory approval, manufacturing consistency, and user acceptance [124], [125], [126].

## Challenges, Limitations, and Knowledge Gaps

We've made great progress, but there are still issues to address. Although many biosensor platforms demonstrate excellent analytical performance under controlled laboratory conditions, relatively few successfully transition from laboratory prototypes to routine field deployment because of technical, regulatory, manufacturing, and economic challenges. One of these issues is validation & standardization. Most new biosensors have been validated and standardized by testing spiked samples. Real world matrices such as soil, whole blood, plant sap etc. can be complex, may interfere, and vary [127], [128]. Matrix interference caused by these complex biological samples may reduce analytical accuracy and increase the likelihood of false-positive or false-negative results under field conditions. There are

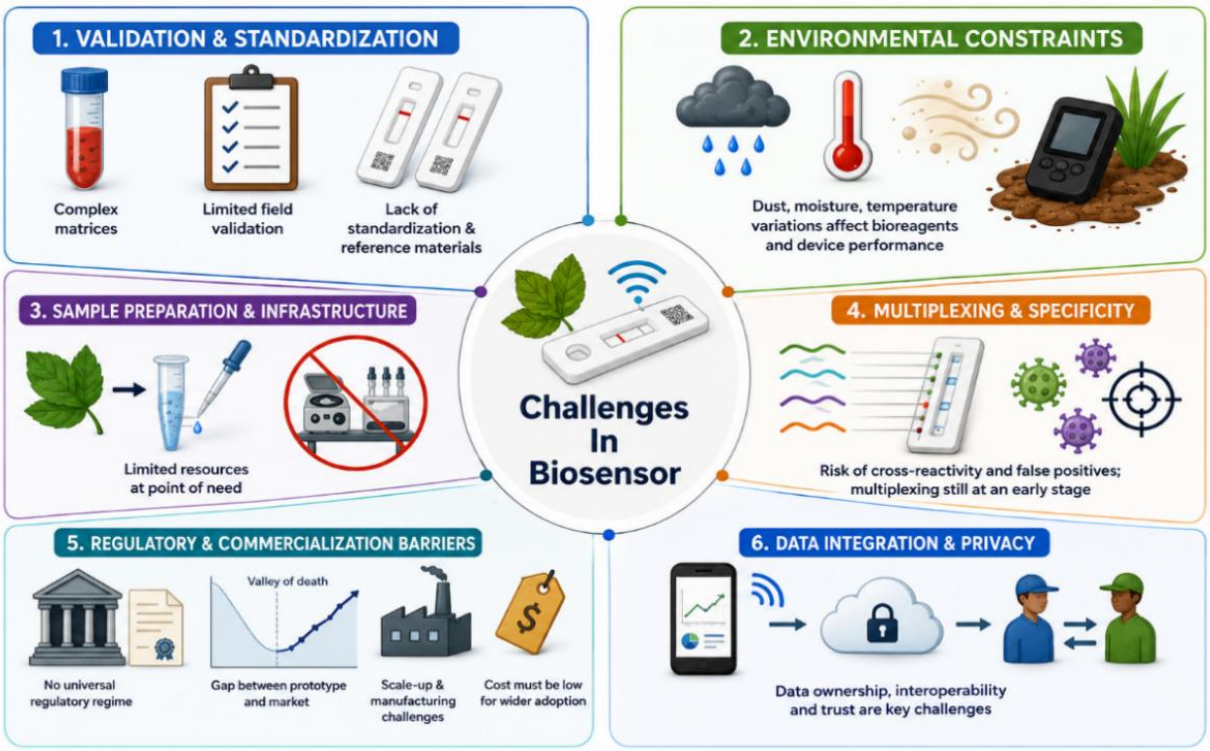



**Figure 4:** Key challenges limiting field deployment of biosensors for plant and animal disease detection.

very few devices that have been formally validated by extensive field tests using gold standard methods. They are not standardized and there are no reference materials, making them incompatible between different platforms. Many biosensor assays do not currently have a regulatory pathway in place for timely approval with regulatory bodies like USDA, EU plant health [129], [130]. Field conditions like dust, moisture and temperature variations pose issues. The activity of bioreagents such as enzymes, antibodies and CRISPR proteins is lost in non-controlled storage. As stated above, humidity and temperature changes can have a significant effect on enzyme stability in the field and therefore on shelf-life. Many assays still need to be refrigerated and shipped on a cold chain. This dependence on cold-chain storage and transportation limits the practical deployment of many biosensors in remote and resource-limited agricultural settings. Miniaturized electronics and optics, too, need to be reliable to make it through a farm or forest [62], [65]. Another limitation is sample preparation and infrastructure. It is still difficult to effectively extract nucleic acids or antigens at source sites. Many biosensor tests, however, assume that the material to be tested is purified, while some microfluidic or paper-based platforms incorporate the sample preparation modules [63]. Devices need to be fully independent as small farms, in general, do not have even the simplest lab resources. Access to laboratory equipment centrifuges and pipettes is often not easy on many farms, especially in the developing countries. But "plug-and-play" designs are essential and difficult to engineer. Furthermore, successful field implementation depends not only on analytical performance but also on adequate user training and simple operating procedures to ensure reliable and reproducible results [131].

The occurrence of co-infection is common particularly in plants. If no efforts are made, one pathogen biosensor may react to another pathogen's biosensor [132]. In principle, CRISPR assays are highly specific, however in complex samples they can lead to false positive due to sub-optimal guide RNAs [133], [134]. The practice of multiplexing (detecting many targets in one test) is at an early stage. The multiplexing functionality built-in by platforms like opto-fluidic arrays or spectral barcoding requires advanced hardware [135]. Also, there are barriers to agricultural diagnostics in the form of regulatory and commercialization barriers.

Agricultural diagnostics do not have a universal regulatory system as there is for diagnostics used in clinical application [136]. It is common for many interesting devices to fail in the "valley of death" between prototype and market due to insufficient funding for field trials and lack of clarity in the approval process. In addition, maintaining manufacturing reproducibility and consistent analytical performance across large-scale production batches remains a major challenge for many emerging biosensor platforms. It is difficult to increase the manufacturing of new nanosensors or cartridges. The

cost needs to be reduced to be competitive with low margin crops or small-holder farmers [137], [138]. It should be noted that smart sensors is just one piece of the puzzle in broader agricultural data ecosystem, where issues surrounding data ownership and accessibility remain major concerns. In addition to engineering challenges, making our data systems safe and inter-operable between platforms, and getting farmers to share data with each other are socio-technical challenges [139], [140]. These restrictions might be overcome in future studies through the collaboration of engineers, biologists, veterinarians, plant pathologists and authorities [62], [140], [141]. The summary of these challenges is given in **figure 4**.

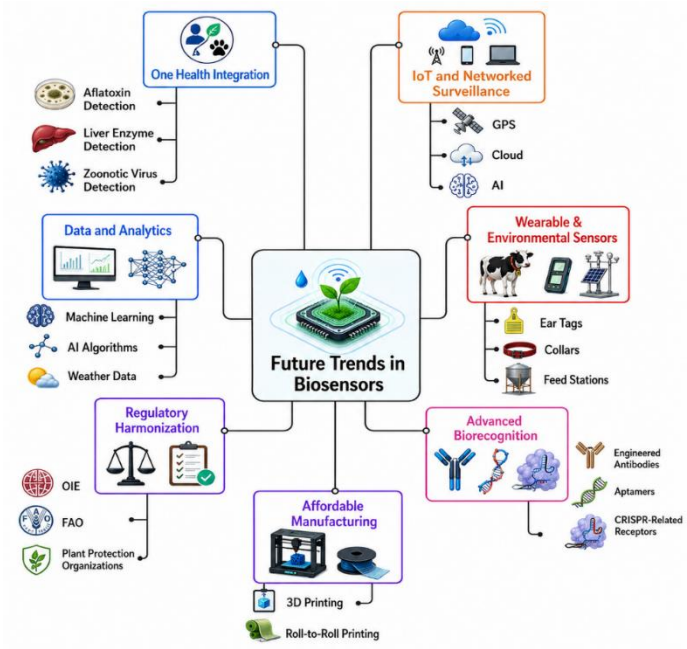
## Future Perspectives

It is expected that biosensors will become a node in an Internet-of-Things (IoT) network. Real-time mapping and decision support are made possible by a combination of GPS, cloud and AI [142]. Imagine farmers having tests available on their smartphones which automatically feed into regional disease models for alerts and action. Networks with the One Health paradigm communicate information on plant, animal and human disease that enables a proactive approach to preventing zoonotic spill overs[142], [143]. For livestock, it is predicted that the number of wearable biosensors (sensors in ear tags, collars or feed/water stations) measuring physiological markers such as temperature, activity, rumen activity will increase, in order to track proxies of infection or stress [144]. Sensor tags can be used to track humidity and other volatile profiles in your fields, or your greenhouses, to see if there is an early warning. Machine learning could fuse multi-source data (e.g. sound sensors detecting coughing in pigs, combined with weather and plant moisture data) to predict disease risk [145].

New bioreceptors like engineered antibodies, high-affinity aptamers, CRISPR-related receptors will improve specificity. Synthetic biology may yield living biosensors: e.g. engineered microbial biosensors that change fluorescence in the presence of a pathogen metabolite [146]. Integration of organ-on-chip technology like plant leaf "organoid" sensors is hypothetical but could provide in situ monitoring of infection [147]. Adoption of additive manufacturing like 3D-printed microfluidics and roll-to-roll printed electronics may dramatically lower production costs for sensors and cartridges. Combined with economies of scale, this could bring diagnostic costs close to consumable levels [148], [149].

Moreover, it will gain more importance in the area of data and analytics, thanks to machine learning. With the ability of AI algorithms to tap into sensor output, weather data, satellite images, and previous outbreak, subtle signals can be detected [150]. An AI could be triggered by an anomaly in the low-priced biosensor and suggest follow-up tests, for example. In the future under the new climate conditions, CRISPR diagnostics will become a part of smart agricultural surveillance networks [69], [151]. The realization of the health of people, animals and the environment will result in intersectoral sensor systems [152]. For example, the detection of aflatoxin in feed (plant side) may be closely associated with the detection of the liver enzymes of livestock [153]. Similarly, animal disease biosensors (e.g. CRISPR test for zoonotic viruses) can be similarly adapted to plant pathogens and vice versa [68]. Figure 3 summarizes future trends. Figure 5 depicts some future trends.

**Figure 5.** The future of smart biosensor ecosystems specifically for animal and plant diseases surveillance.




## Conclusions

Advancements in biosensor technologies have enabled the early detection of diseases in plants and animals. With the advancement of basic field tests into multiplex assays, a number of platforms have emerged, ranging from enzyme electrodes to strips the size of a fingernail and decorated with gold nanoparticles to CRISPR-based chips and smartphone readers. Particularly, innovations in recent years have speeded up the sensitivity and usability. The advances will bring real-time diagnostics to agriculture and veterinary health. It is not easy to translate laboratory prototypes into reliable field-deployable products. It is essential to develop biosensor reagents that are fully validated according to international quality and regulatory standards. If we can

get over these hurdles, the benefits will be evident. In the future, we are going to see biosensors that are embedded in "smart farming" systems. These will be connected devices which will track the condition of crops and herds at all times. Finally, it is forecasted that the biosensors will be widely adopted in agriculture and veterinary medicine.

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The authors declare no conflict of interest.

## Author Contributions:

Faizan-e-Mustaffa (FEM) devised the review topic and designed the overall study. FEM and Munawar Ali (MA) performed the literature search, literature screening, and data extraction. Hafiz Muhammad Sultan (HMS) contributed to the interpretation and synthesis of the literature. FEM prepared the initial manuscript draft. Syeda Areej Imran (SAI) and Tehseen Sajid (TS) assisted with figure preparation, data curation, and proofreading. FEM and HMS critically revised the manuscript for important intellectual content and supervised the study. All authors reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

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