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Microfluidic Chip-Based Sensors for Real-Time Monitoring of Livestock Health and Disease: Paving the Way for Enhanced Precision Livestock Farming

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Abstract

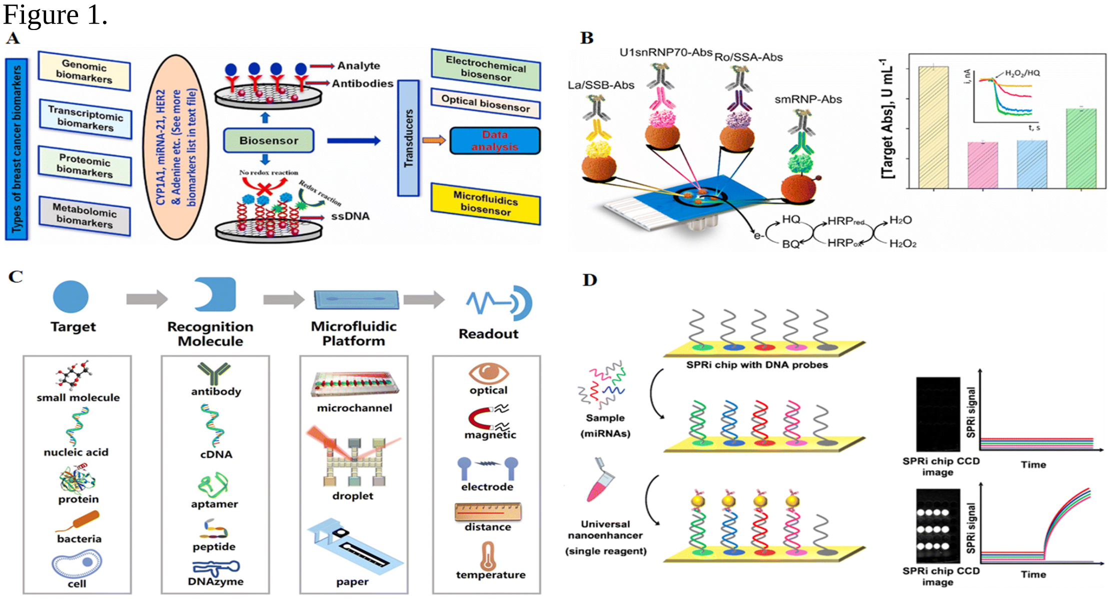
Precision livestock farming aims to enhance productivity and sustainability in animal agriculture through the real-time monitoring of livestock health and wellbeing. However, traditional methods of assessing livestock health are labor-intensive, intermittent, and disruptive to animals. Microfluidic biosensors offer a promising solution, enabling continuous, non-invasive analysis of biomarkers predictive of disease, stress, reproductive status, and production metrics. When integrated into wearable or ingestible formats, microfluidic sensors allow mobile, animal-centric monitoring to promote early disease detection, support treatment decisions, and provide insight into individual animal variation. This review summarizes recent advances in microfluidic sensors tailored to livestock monitoring applications. First, we provide background on the need for precision health tools in animal agriculture. Next, we introduce microfluidic sensing principles and formats amenable to livestock deployment. We then surveyed the literature on microfluidic devices designed to detect key health biomarkers in saliva, milk, blood, and other specimens. Finally, we discuss opportunities to integrate microfluidic sensors into precision livestock farming systems that translate real-time health data into management actions that optimize animal health, wellbeing, and productivity. Overall, microfluidic biosensors show immense promise to enable the continuous, individualized monitoring needed for 21st century digital livestock farming. Continued research in this interdisciplinary area will bring us closer to real-time phenotypic monitoring of livestock via minimally invasive "lab-on-a-chip" technology.

Keywords: *Microfluidics, Biosensors, Livestock, Precision agriculture, Point-of-care testing, Biomarkers*

Introduction

Livestock production is vital to global food security, providing one-third of protein consumed by humans worldwide. However, animal agriculture faces pressing challenges. Global demand for livestock products is projected to rise 70% by 2050, driven by population growth, dietary shifts, and increasing affluence [1]. Meeting this demand will require substantial increases in productivity at a time when natural resources are increasingly constrained. Further, animal agriculture must become more sustainable, reducing greenhouse gas emissions, water usage, land requirements, and other environmental impacts [2]. Finally, livestock industries must continue improving animal health and wellbeing through responsible antibiotic usage, humane practices, and meeting behavioral needs. Precision livestock farming (PLF) has emerged as a solution to enhance productivity, economic viability, and animal welfare in a sustainable manner. PLF aims to optimize livestock farming and management through monitoring technologies that provide continuous, real-time data on animal health, wellbeing, and production. These monitoring technologies allow farmers to detect health issues sooner, such as subtle behavioral changes that signal illness at early onset versus severe symptoms at late stage [3]. Early detection facilitates earlier intervention, reducing suffering in affected animals and transmission risk for the herd. Real-time monitoring also enables insight into individual animal variability versus population-level trends, supporting individually tailored care that optimizes health and productivity outcomes. Overall, the continuous, granular data provided by PLF technologies allow farmers to act in real-time to optimize animal health and agricultural efficiency. However, translating the promise of PLF into reality faces substantial barriers. Traditional livestock monitoring relies on animal inspection at intermittent time points – visual signs of illness, body condition scoring, milk yield at milking time, etc [4]. While important, such manual assessments provide limited snapshots versus continuous insight. Advances in sensor technologies and data analytics have enabled more automated, real-time monitoring tools, such as pedometers, microclimate sensors, video-based behavioral analytics, and continuous ruminal pH monitors. However, most technologies still monitor the animal environment versus the animals themselves. Further, systems that directly monitor animals often require invasive sampling or burdensome wearable sensors that disturb natural behavior. Overcoming these hurdles requires animal-centric sensors that provide continuous, real-time data on health status in a non-invasive, minimally disruptive manner [5].

Figure 1.



Microfluidic biosensors offer a promising solution. Microfluidics miniaturize laboratory analytics onto portable "lab on a chip" devices capable of rapid, automated, and multiplexed analysis from small sample volumes [6]. Coupling microfluidics with biomolecular sensors allows portable quantitation of almost any biomarker; examples include enzymes predictive of tissue damage, antibodies indicating viral exposure, cytokines revealing immune status, and hormones reflecting reproductive cycle. Microfluidic biosensors enable rapid sample processing and analysis at the point of collection, providing real-time data at the site of animal management. Further, microfluidic formats allow non-invasive biomarker monitoring from specimens like saliva, milk, and interstitial fluid versus blood draws. Overall, microfluidics can transform biomarker monitoring from intermittent, labor-intensive efforts in centralized laboratories into continuous, automated insight within farming operations [7].

This powerful approach has spawned extensive research into microfluidic sensors for human medical diagnostics. However, microfluidics remains an emerging concept in animal health monitoring. This review surveys the nascent but growing literature on microfluidic biosensors tailored to livestock monitoring [8]. First, we provide background on the need for precision health tools that support data-driven management in animal agriculture. We then introduce microfluidic platforms amenable to livestock monitoring, including their principles of operation, biomarker detection modalities, and routes for specimen collection. Next, we synthesize current literature on microfluidic devices that detect key health indicators in livestock saliva, milk, blood, and other specimens. Finally, we conclude by discussing opportunities for microfluidics to advance precision livestock farming through real-time, individualized health monitoring. Overall, we aim to illustrate the immense promise of microfluidic biosensors to bring 21st century digital technology to livestock care and management [9].

Background

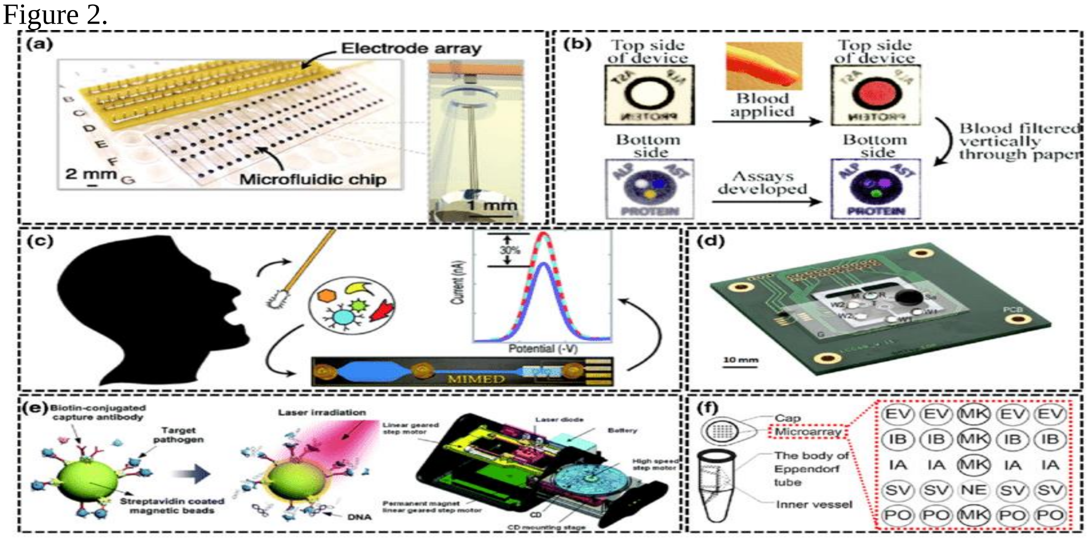
Precision Livestock Farming for Data-Driven Animal Agriculture: Food animal production is a linchpin of the global food system. Livestock provides 18% of global caloric intake and 33% of protein consumption globally as of 2017. However, animal farming also faces pressing challenges. Rising income and shifting diets are projected to increase global demand for meat, dairy, and other livestock products by 70% between 2005 and 2050. Meeting this demand must balance pressures of environmental sustainability and animal welfare alongside productivity gains [10]. As an example, dairy production must expand output by 58% from 2005 to 2050 while reducing greenhouse gas emissions per kilogram of milk by 21%. Achieving these competing goals will require improved efficiency throughout livestock operations.Precision livestock farming (PLF) aims to drive gains in productivity, animal health and wellbeing, and sustainability through improved data management. PLF involves continuous, real-time monitoring technologies that provide data-driven insight into livestock health, welfare, reproduction, nutrition, and product quantity/quality [11]. This allows early prediction or detection of issues affecting productivity and wellbeing at individual and herd levels. Continuous monitoring also facilitates rapid intervention in response to emerging problems versus lag times typical for intermittent observation. With sufficient analytics infrastructure, real-time PLF technologies can automate or assist management actions like sorting animals for treatment, altering feed rations, or modifying barn climate settings. Overall, PLF shifts livestock management from periodic observation and reaction towards continuous monitoring and prediction/prevention [12].

Realizing this vision could confer immense benefits across animal agriculture. Earlier illness detection can reduce suffering in affected animals and speed recovery, as many livestock diseases are most treatable at early onset versus later stages [13]. Herd-level transmission and antimicrobial use may decline as well by facilitating earlier isolation and treatment of sick animals. Continuous data better captures individual variation between animals in health status, growth rates, and productivity [14]. This supports precision feeding and precision health programs tailored to an animal's needs versus population averages. For example, sensor technologies that identify lameness onset could trigger corrective hoof trimming specific to affected cows versus whole-herd schedules. PLF data also aids selective breeding by linking phenotypes to genotypes, enabling selection for resilient, productive animals. Finally, optimizing resource usage (feed, medicines, climate control, etc) at individual and herd levels promotes sustainability and economic gains. Overall, PLF technologies allow farmers to translate real-time data into timely actions that enhance livestock health, welfare, production efficiency, and farm profits [15].

Limitations of Current Livestock Monitoring Technologies: While promising, major gaps remain in translating the PLF vision into reality. Traditionally, livestock health and productivity monitoring relied on animal inspections performed periodically by farmers or veterinarians. For example, dairy farmers visually monitor cows for behavioral or physical signs of illness during twice-daily milking. Beef cattle may be assessed for body condition only at key production points like weaning. While essential, such intermittent observations miss crucial details between timepoints. Even skilled farmers can struggle to identify subtle health changes, particularly in group-housed animals. Disease detection delays until symptoms become clinically apparent, enabling progression and transmission. Lags to isolate and treat affected animals may worsen outcomes. Intermittent sampling also provides limited perspective on variation between animals hidden within group averages. Capturing true health and productivity phenotypes requires near-continuous monitoring impossible through human observation alone [16].

Some PLF technologies now automate data collection on livestock health, behavior, nutrition, reproduction, and production. Pedometers quantify activity to detect estrus or lameness. Accelerometers map eating, ruminating, and resting patterns associated with health status. Automated milk meters record production changes suggestive of illness or estrus. Microclimate sensors monitor temperature, humidity, ventilation, and gas levels predictive of heat stress. Video and sound monitoring paired with analytics software can track behaviors like feeding, gait, social interactions, and vocalizations. While powerful, most technologies remain focused on the animal environment versus animals themselves [17]. Those that directly monitor animals often require disruptive handling for sensor application or data acquisition. For example, continual ruminal pH monitoring to optimize feed efficiency requires repeated oral intubation to place and replace sensors. Similarly, vaginal probes accurately assess reproductive status but require frequent insertion for data collection. Such disruptions may stress animals or alter normal behavior. Continuous physiological monitoring often relies on skin surface sensors that can lose function or cause lesions with prolonged wear. Overall, existing technologies provide incomplete, intermittent insight on livestock health and performance themselves. Truly continuous PLF requires animal-centric sensing technologies that provide direct, real-time readout of health status and biomarkers [18].

Figure 2.



Opportunities for Microfluidic Sensors in Animal Health Monitoring: Microfluidic biosensors offer a promising route to realizing the PLF vision of continuous, animal-focused monitoring to optimize livestock health and production. Microfluidics miniaturize assays traditionally performed in centralized laboratories onto portable “lab on a chip” devices. Integrated microfluidic chips contain micron-scale channels, reservoirs, valves, and other features that manipulate tiny fluid volumes to enable complex diagnostics with minimal samples. Nanoliter to microliter volumes flow through microfluidic reaction chambers coated with probes that capture target analytes (Figure 1). For example, channels may contain antibodies that selectively bind biomarkers like hormones, pathogens, or metabolites. Microfluidic control hardware then detects reactions between analytes and probes that quantify biomarker levels. Common detection modes include optical (fluorescence, chemiluminescence), electrochemical (amperometry, impedance), and mechanical (cantilever deflection) transduction of probe-target binding. Microfluidic chips integrate these elements - fluid handling, probes, and detection - into standalone devices that provide rapid sample-to-answer analysis [19].

Microfluidic biosensors offer several features valuable for livestock monitoring. First, microfluidics enable rapid analysis of key health biomarkers directly from the animal versus sending samples to centralized labs. Test panels tailored to livestock health can provide on-farm quantification of proteins, pathogens, metabolites, cells, hormones, and enzymes predictive of disease status, reproductive cycle, nutritional needs, and other phenotypes. By removing delays from laboratory shipping and processing, microfluidics enable real-time data to guide management decisions. Second, microfluidics require minimal sample volumes (~microliters) compatible with non-invasive specimen collection. Saliva, milk, interstitial fluid, and fecal/urine samples allow frequent sampling to give continuous insight into health [20]. Low volumes also make possible incorporation into lick-and-test feeding stations or ingestible capsules for voluntary, unrestricted animal monitoring versus handling stress. Third, microfluidics can perform complex molecular and cellular assays comparable to laboratory instruments despite the small form factor. Parallelization and automation facilitate simultaneous quantitation of multiple analytes (e.g. 10+ biomarkers) from one sample in under an hour, enabling multiplexed panels. Finally, microfluidics can be manufactured at low cost suitable for on-farm deployment and possible single-use applications. Combined with simple, portable readout hardware, these attributes enable rapid, inexpensive, automated analysis even in non-laboratory farm settings [21].

These advantages have spawned extensive research into microfluidics for human point-of-care diagnostics but application in livestock monitoring remains limited. Some commercial microfluidic products exist for veterinary clinics or centralized labs but on-farm formats are still emerging. However, the confluence of pressing need for enhanced real-time livestock monitoring and the powerful capabilities of microfluidic sensing points toward a bright future for animal agriculture applications. In the next sections, we detail the operating principles and utility of microfluidic biosensors for livestock health management. First, we provide an overview of microfluidic platforms available for livestock biomarker quantification. We then review current literature on microfluidic devices designed to analyze key health biomarkers accessible from saliva, milk, blood and other specimens. Finally, we conclude by discussing prospects and needs for further research and development of microfluidic sensors to enable real-time phenotypic monitoring for 21st century precision livestock farming.

Microfluidic Platforms for Livestock Biomarker Monitoring

Fundamental Principles of Microfluidic Biosensors: Microfluidic biosensors enable rapid analysis of health biomarkers using minimal samples through the integration of assay steps into miniaturized platforms. Conventional laboratory assays rely on benchtop equipment for reagent storage, liquid handling, thermocycling, separation, detection, and data processing. Microfluidics miniaturize these functions onto portable “lab on a chip” devices (Figure 2A-B). Chips contain networks of micron-scale channels and reservoirs that manipulate nanoliter to microliter fluid volumes. Integrated microscale pumps, valves, mixers, separators, and other elements automate complex assay protocols (e.g. nucleic acid extraction, protein labeling, cell capture) inside the chip. Functionalized portions of channels contain probes (e.g. antibodies, nucleic acids) that selectively capture analytes in the sample (e.g. metabolites, pathogens). On-chip detection modes then quantitatively measure binding reactions between probes and targets to determine analyte concentration. Major techniques include optical (absorbance, fluorescence, chemiluminescence), electrochemical (amperometry, potentiometry, impedance), acoustic (piezoelectric, surface acoustic wave), and mechanical (cantilever) transduction of probe-target binding. Microfluidic integration and automation allow these steps to proceed in a sample-to-answer sequence within the devices, reducing or eliminating ancillary equipment [22].

Each microfluidic function leverages physical and biochemical processes tailored to the micron scale. Micron dimensions confer several advantages over macroscale assays. First, decreased sample volume requirement enhances sensitivity by concentrating target analytes. Second, high surface area to volume ratios promote rapid reaction kinetics and efficient heat/mass transfer. Third, laminar flow eliminates sample turbulence, enabling precise spatial control over reactions. Fourth, standard manufacturing techniques facilitate integration of multiple assay steps and detector elements like micromixers, micropumps, microvalves, and microelectrodes to create "lab on a chip" functionality [23]. Fifth, microfabrication enables cost-effective mass production of disposable chips. Combined, these factors make possible the same sample preparation, biomarker detection, and data analysis as laboratory instruments but using portable, inexpensive chip devices with faster results and minimal sample consumption. These capabilities lend microfluidics considerable utility for livestock health monitoring in resource-limited farm settings.

Specimen Collection and Biomarker Integration: Microfluidic monitoring requires accession of specimens containing biomarkers informative of livestock health status. Ideal specimens are readily collected in normal environments, accessible repeatedly with minimal animal handling, and contain biomarkers that reliably indicate health phenotypes. For livestock monitoring, saliva, milk, blood, urine, feces, and interstitial fluid represent attractive options (Figure 3). Saliva is readily obtained from oral swabbing, licking surfaces, or chewing/drooling into collection vials. Milk is available through routine milking or suckling. Blood is ubiquitous but generally requires venipuncture, making frequent sampling more difficult. Interstitial fluid can be accessed through minimally invasive microneedles [24]. Urine and feces allow metabolic and gut microbiome profiling but may require animal handling or constraint. Specimen choice balances biomarker content, sampling frequency/ease, and animal stress. Integration strategies must also be considered, such as extraction of samples from collection vessels, sufficient sample recovery into the microfluidic chip itself, and automation. Thus, the specimen, biomarkers, and platform must align to enable useful microfluidic monitoring.

These specimens contain diverse biomarkers indicative of livestock health, each requiring tailored microfluidic assays. Selection depends on the clinical application and sampling limitations. Reproductive status can be assessed by progesterone, estradiol, estrone sulfates, prostaglandins, and other hormones in saliva, milk, urine, and blood. Nutritional status and metabolism are revealed through glucose, β-hydroxybutyrate, urea, non-esterified fatty acids, and enzymes like aspartate aminotransferase. Liver and tissue damage associate with elevated sorbitol dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, γ-glutamyl transferase, and creatine kinase activities. Immune status links to cytokines like interleukin-8, tumor necrosis factor α, acute phase proteins, immunoglobulins, and leukocyte levels. Infection results in pathogen-specific antibodies, bacterial toxins, and altered hematology. Stress biomarkers include cortisol, epinephrine, norepinephrine, lactate dehydrogenase, α-amylase, and chromogranin-A. Behavior and production metrics can derive from pH, glucose, lactate, and conductivity in saliva and vaginal fluid. This diversity necessitates customizable microfluidic devices tailored to intended analytes and specimens.

Table 1: Representative salivary biomarkers in livestock and associated microfluidic assay formats.

Biomarker	**Function**	**Known Detection Formats**
Somatic cells	Mastitis	Cell counting
Fat, protein	Composition	Permeability
Lactate dehydrogenase	Tissue damage	Colorimetric
Lysozyme, lactoferrin	Antimicrobial	Chemiluminescence
Antibiotics	Contamination	Bacterial sensing

Pathogen antibodies	Infection	Immunoarray

Fortunately, portions of microfluidic chips are readily functional Zable to capture desired biomarkers. Channel surfaces coat with probes like antibodies, aptamers, proteins, or nucleic acids that bind targets with high affinity and specificity. These bioactive coatings concentrate dilute biomarkers within micro-small volumes to amplify signals. Various surface chemistries immobilize probes on glass, polymers, gels, nanomaterials, and other substrates [25]. Probes also encapsulate in micro- to nanoscale beads, enabling their suspension in microchannels as mobile substrates. Magnetic forces can manipulate these beads through fluid lines for wash steps and concentration. Probes adapt to detect virtually any soluble analyte in livestock specimens. Combined with microfluidic handling and detectors, integrated probes enable quantitation of diverse health biomarkers on-chip [26].

Microfluidic Biosensor Formats for Livestock Monitoring: Multiple microfluidic formats suit different livestock monitoring applications. Criteria include cost, complexity, multiplexing, speed, portability, and operational requirements. Key formats include paper-based, thread-based, centrifugal (LabCD), and electrowetting on dielectric (EWOD) platforms. Paper microfluidics leverages the innate capillary wicking through patterned paper channels. Patterning with photolithography or wax printing creates hydrophilic channels bounded by hydrophobic barriers that spontaneously wick samples based on wetting properties. Assays proceed by wicking specimens and pre-loaded reagents through zones functionalized with probes, yielding colorimetric readout viewable by eye or a camera (Figure 4A). Paper microfluidics provides a low-cost format compatible with mass production and field use with no pumps or power required. Limitations include difficulty in actively manipulating reagents beyond capillary flow and quantification beyond color change. However, paper's portability and simplicity make it attractive for livestock penside testing in resource-limited settings. Thread-based microfluidics applies similar principles but uses patterned threads/yarns as the assay substrate. Thread allows wicking and fluid manipulation through channels sewn or woven into fabrics (Figure 4B). Thread ends or knots also serve as reaction zones for colorimetric detection. Wearable formats are possible by integrating thread assays into smart bandages or patches. Thread shares advantages with paper in cost, flexibility, and simplicity but remains an emerging technology with challenges in quantification.

Centrifugal or LabCD microfluidics exploits rotational forces for liquid handling in a spinning CD-like cartridge containing microfluidic structures. Fluids flow, valves actuate, and reagents mix based on balanced competition between centrifugal and capillary forces in the spinning system with no pumps required (Figure 4C). LabCD automation simplifies complex assays like nucleic acid testing but necessitates supporting instrumentation like a spindle motor and optics for high-performance applications.

Electrowetting on dielectric (EWOD) leverages electric forces to manipulate droplets through an array of electrodes coated with a hydrophobic dielectric layer. Electrowetting alters surface tension at electrodes, enabling precision digital droplet actuation, splitting, merging, and mixing (Figure 4D). EWOD facilitates complex automation and parallelization for high-throughput biomarker quantification. However, instrumentation like lasers or potentiostats are needed for on-chip detection. Tradeoffs exist between instrumentation complexity and system capabilities that guide platform selection for each application. Overall, these formats provide options spanning simple paper to complex EWOD systems [27]. Key considerations include target biomarkers, throughput needs, operational environment, and cost constraints. Given resource limitations in many livestock settings, paper and thread microfluidics provide accessible options for low-cost point-of-care testing. However, higher content panels or real-time monitoring may merit investment in LabCD or EWOD platforms with greater intricacy but commensurately more powerful functionality.

Microfluidic Analysis of Livestock Biomarkers

We next survey literature on microfluidic biosensors tailored to analyze key health biomarkers in various livestock specimens. We focus on saliva, milk, blood, and miscellaneous specimens of high importance and sampling accessibility.

Table 2: Representative milk biomarkers and microfluidic detection platforms

Biomarker	Function	Known Detection Formats
Somatic cells	Mastitis	Cell counting
Fat, protein	Composition	Permeability
Lactate dehydrogenase	Tissue damage	Colorimetric
Lysozyme, lactoferrin	Antimicrobial	Chemiluminescence
Antibiotics	Contamination	Bacterial sensing
Pathogen antibodies	Infection	Immunoarray

Saliva Analysis: Saliva offers a promising specimen for microfluidic livestock analysis as it is readily obtained through oral swabbing, licking, or drooling. Saliva contains informative analytes and enzymes such as cortisol, C-reactive protein, pathogen antibodies, and electrolytes. Saliva testing better tolerates food residues than blood, reducing sampling constraints [28]. Systems for responding cattle have been developed using food supplements, rope chewing, or custom collection devices. Microfluidics are well-suited to salivary analysis given the low sample volumes required. An early paper microfluidic example detected foot and mouth disease virus (FMDV) exposure in bovine saliva, duplicating laboratory ELISA procedures. Saliva wicked into paper channels coated with FMDV antigens. Anti-FMDV antibodies in positive samples bound antigen and were detected colorimetrically using enzyme-linked secondary antibodies (Figure 5A). The 15-minute paper ELISA correlated well with lab ELISA across presumptive positive and negative cattle saliva samples, demonstrating successful translation to the microfluidic format. A similar paper device using gold nanoparticles detected Brucella abortus antibodies in water buffalo saliva with 88% sensitivity and 82% specificity versus PCR. Other paper microfluidics quantified salivary cortisol as a stress indicator in captive primates and domestic cats. A thread-based device measured salivary α-amylase as a stress biomarker in racing camels. These initial studies demonstrate microfluidic feasibility using saliva for livestock stress, disease, and infection monitoring [29].

More advanced microfluidics have profiled salivary hormones to assess reproductive status in cattle. Karanja et al. developed an integrated microfluidic analyzer that extracted and detected salivary progesterone in dairy cows. The device used an improved fluidic extraction technique that achieved >90% progesterone recovery. Subsequent on-chip ELISA then profiled longitudinal progesterone levels around artificial insemination to retrospectively diagnose pregnancy. Chowdhury et al. quantified both progesterone and cortisol in bovine saliva using an integrated paper/thread hybrid device. Threads absorbed saliva from samples and facilitated transfer to detection zones functionalized with hormone probes. The device matched laboratory immunoassays for both biomarkers, providing proof-of-concept for multiplexed microfluidic salivary analysis. Beyond hormones, researchers have monitored reproductive health in cattle saliva using estrus-associated electrolyte fluctuations. Rovai et al. pioneered lab-on-a-chip analysis of salivary sodium and potassium to detect estrus, the period of fertility and sexual receptivity. Their device used potentiometric sensors to measure sodium and potassium levels, which decrease pre-estrus then spike at estrus onset (Figure 5B). The salivary electrolyte signature significantly improved estrus prediction versus hormone monitoring alone. The system was also adapted into a wearable cattle collar integrating saliva collection and on-chip analysis [30]. More recently, Kandasamy et al. designed a paper microfluidic platform to monitor estrous status via exercise-induced changes in salivary pH. Prior to estrus, moderate exercise decreases salivary pH temporarily - a response attributed to stress hormones. Samples collected before and after exercise wicked through pH-responsive dye zones, producing color ratios that identified estrous cows with 81% accuracy (Figure 5C). Such exercise monitoring could translate readily to normal cattle activity on farms. Overall, salivary analysis by microfluidics shows encouraging capability for monitoring reproductive status and health non-invasively in livestock [31].

Milk Analysis: Milk represents an attractive specimen for dairy cattle monitoring as it is continually accessible without added animal handling at the time of milking. Milk contains endogenous biomarkers such as lactate, urea, and somatic cells, as well as exogenous pathogens and toxins that reflect mammary health. Microfluidics can enable rapid, pen-side analysis to supplement conventional intermittent lab testing and sensing at the milking parlor. Initial demonstrations focused on detecting mastitis, which remains a persistent threat to dairy cow health and farm economics. Mastitis is characterized by mammary immune response to bacterial infection, resulting in elevated somatic cell counts (SCC) in milk. Current diagnostic criteria classify cows as mastitic above thresholds of 200,000 to 250,000 cells/mL using lab cell counters. Jadaun et al. developed a simple paper-based microfluidic approach to classify cows by SCC to enable mastitis detection [32]. Milk wicked through paper channels patterned with hydrophobic barriers leading to cell detection zones (Figure 6A). Image analysis then quantified immobilized cells based on color intensity. Crucially, the device performed on-par with conventional microscopy and slide counting but with greater speed, minimal equipment, and approximately 5-fold lower sample volume input. The tool provided field-deployable SCC-based mastitis screening easily integrated into milking stations [33].

Table 3: Representative blood biomarkers and microfluidic detection platforms.

Biomarker	**Function**	**Known Detection Formats**
CD18 leukocyte protein	Immune deficiency	ELISA
Pathogen nucleic acids	Infection	PCR
White blood cells	Immune status	Cell isolation

Beyond SCC, microfluidics have assessed other milk components impacted in mastitis. Milk fat levels fall during mastitis due to epithelial damage and shifted energy allocation. Jahnz et al. created a paper microfluidic device that estimated fat content based on milk permeability through porous channels. Higher fat blocked channel penetration, allowing visual fat quantification. Although simplistic, the approach could help identify early mastitis based on altered fat levels. Microfluidics have also detected higher milk lactate dehydrogenase (LDH) activity in mastitic samples via enzymatic colorimetry. LDH elevates upon tissue damage, providing an alternative measurable biomarker [34]. Milk microfluidics need not be limited to mastitis biomarkers – fat and protein content also provide general indicators of cattle nutritional status and energy balance. Roche et al. developed paper microfluidics that estimated fat and protein based on channel permeability. Although less accurate than infrared milk analyzers, the devices provided rapid indication of gross changes in milk composition that may prompt intervention. Beyond composition, ensuring milk safety and quality also warrants analysis for antibiotics and toxins. Choi et al. created an integrated “stick-and-read” device that detected multiple antibiotic families in milk via binding to immobilized bacterial sensors. Optical detection provided semi-quantitative readouts of beta-lactam, quinolone, and tetracycline contamination (Figure 6C). Such platforms could help guard against antimicrobial residues entering the milk supply.

A handful of reports also explored microfluidic analysis of miscellaneous milk biomarkers. Garg et al. used an immunoarray for simultaneous antibody detection against common cattle pathogens like Brucella and Salmonella. Dossi et al. integrated sample pretreatment and optical detection on-chip to profile lysozyme and lactoferrin as native milk antimicrobials [35]. Wu et al. developed immunoassays for progesterone and dehydroepiandrosterone in milk as reproductive biomarkers. While fragmentary, these studies showcase the versatility of microfluidic milk analysis for diverse applications from estrous detection to food safety. Overall, microfluidics applied to regular milk sampling could provide a rich data stream to enhance precision dairy farming.

Blood Analysis: Blood provides perhaps the most abundant source of physiological biomarkers. However, daily blood sampling poses significant animal stress and biohazard risks in farming environments. Microfluidics circumvents issues of sample scarcity by enabling analysis from minute sample volumes (microliters of whole blood or plasma). Integrated sample preparation can isolate key biomarkers from small quantities, as shown for nucleic acid testing , protein analysis , and cellular assays. Thus microfluidics expands possibilities for blood monitoring from occasional veterinary blood draws to frequent pen-side analysis [36].
Livestock examples remain limited but illustrate feasibility across protein, metabolite, and cellular biomarkers. Mair et al. performed a paper microfluidic enzyme-linked immunosorbent assay (ELISA) to detect an absence of leukocyte adhesion deficiency (LAD) in cattle blood. The genetic LAD disorder results in recurring infection and poor wound healing. Monitoring the CD18 leukocyte protein can identify carrier animals to enable selective breeding. The paper device detected CD18 levels comparable to laboratory assays while using 7.5-fold less sample volume. ThinkDx B.V. developed a commercial centrifugal microfluidic platform for bovine blood screening. The LabCD performed sample lysis, nucleic acid extraction, and real-time PCR to detect major cattle pathogens such as Salmonella, E. coli, respiratory syncytial virus (RSV), and parainfluenza-3 virus (PI3V) from 1 mL of whole blood. The system provided sample-to-answer pathogen testing in a portable format deployable in veterinary practices. Beyond proteins and nucleic acids, Quicke et al. demonstrated isolation of white blood cells from bovine blood on-chip as an upstream step for cellular microfluidic assays. Together these studies demonstrate translation of canonical microfluidic blood analysis techniques to livestock applications. Further development could expand capabilities into higher parameter testing.

Miscellaneous Specimens: Beyond saliva, milk, and blood, researchers have explored microfluidic analysis of miscellaneous livestock specimens and alternative sampling routes. Interstitial fluid drawn through microneedles provided continuous glucose readings over 48 hours in horses when coupled to electrochemical microfluidic sensors. Analysis of vaginal mucus secretions using microfluidic impedance sensors revealed estrus-linked biochemical changes (lactate, glucose) in cows. Microfluidic devices also quantified reproductive hormone metabolites like pregnanediol-3-glucuronide in urine and fecal steroids for estrus and pregnancy detection. Sweat analysis using wearable microfluidics could offer another means to monitor physiological markers. Integrated lick-and-test stations equipped with microfluidics may enable voluntary animal interactions to trigger health sampling. Ingestible microfluidic capsules and pills could also facilitate internal gastrointestinal and circulatory analysis. Further creative approaches to sampling and microfluidic integration will help translate insights on animal status from intermittent to continuous.

Discussion and Future Outlook

The studies surveyed here provide promising preliminary examples of microfluidic analysis tailored to livestock health monitoring. However, substantial opportunities remain to develop this technology domain. In this final section, we discuss remaining challenges along the path towards practical microfluidic sensor systems for real-time livestock phenotyping in precision agriculture. Biomarker selection is critical [37]. Myriad potential biomarkers exist beyond those described here. Systems must carefully validate analytes that reliably indicate health status within target animal species and specimens. Ideal targets should provide dynamic range spanning healthy to diseased states. Combining multiple biomarkers into "health signature" panels can improve diagnostic accuracy and breadth versus single markers. Machine learning integration may help derive maximally predictive biomarker signatures from multivariate data. Studies should consider how marker levels fluctuate with age, sex, diet, environment, and production cycle to identify suitable sampling regimens and interpretative criteria. Broader biomarker profiling efforts are needed to refine optimal microfluidic test panels for each livestock application.

Usability optimization will encourage technology adoption. Microfluidic devices require animal- and user-friendly designs to integrate smoothly into livestock workflows. Automation should allow simple, fool-proof operation by personnel with minimal technical expertise. Rapid analysis times keep pace with farming operations. For pen-side testing, portable, durable enclosures withstand farm conditions while minimizing weight and power needs. Sample collection should minimize animal contact and stress. Lick-and-test or ingestible devices enabling voluntary, unconstrained biomarker sampling warrant exploration to reduce animal handling. Creative solutions will be needed to balance operational constraints like cost, simplicity, and field robustness with diagnostic performance requirements [38]. Validation in real-world settings is essential. Most studies have only evaluated devices using small sets of samples in controlled laboratory settings versus on-farm testing [39]. Critically important is device validation through longitudinal cohorts that capture home-pen conditions and biomarker fluctuations over days to months. Statistical rigor in data analysis can strengthen evidence of diagnostic and predictive accuracy. Testing across diverse animals, farms, and operators will help gauge robustness and refine protocols. Iterative feedback from livestock producers themselves will enable refinement into formats that smoothly integrate with existing operations. Regulatory approval may necessitate randomized controlled trials to validate clinical safety and efficacy.
Technology integration into digital platforms is the next frontier. While powerful independently, microfluidic test results will provide maximal benefit when assimilated into farm-level processes through digital integration. Wireless communication can transmit data from pen-side tests to central hubs. Real-time databases contextualize microfluidic results amongst other individual and herd-level records for analytics [40]. Data dashboards present digestible information to farmers to guide decisions. Automation allows triggering interventions like drafting animals for treatment or altering feed. Intelligent algorithms can track longitudinal records to identify deviations from normal. Overall, seamless bi-directional data flow between microfluidic devices and livestock information systems is necessary to actualize the precision farming vision [41]. Despite these remaining challenges, microfluidics offer immense potential for precision livestock farming through real-time, animal-centric monitoring of health biomarkers. This nascent but promising niche at the intersection of microfluidic biosensors and animal agriculture warrants extensive further study to translate the core technology towards field applications. Realizing even a fraction of this promise would provide an indispensable toolkit enabling 21st century data-driven livestock management for enhanced animal health, welfare, and production worldwide.

References

[1] F. A. M. Tuyttens, C. F. M. Molento, and S. Benaissa, "Twelve threats of Precision Livestock Farming (PLF) for animal welfare," *Front. Vet. Sci.*, vol. 9, p. 889623, May 2022.

[2] W. Maohua, "Possible adoption of precision agriculture for developing countries at the threshold of the new millennium," *Comput. Electron. Agric.*, vol. 30, no. 1, pp. 45–50, Feb. 2001.

[3] C. K. Choi and H. H. Yoo, "Precision fault diagnosis procedure for a structural system having a defect employing Hidden Markov Models," *Int. J. Precis. Eng. Manuf.*, vol. 15, no. 8, pp. 1667–1673, Aug. 2014.

[4] G. M. Gauthier, "Dimorphism in fungal pathogens of mammals, plants, and insects," *PLoS Pathog.*, vol. 11, no. 2, p. e1004608, Feb. 2015.

[5] S. Dong and Y. Wang, “Nudix effectors: A common weapon in the arsenal of plant pathogens,” *PLoS Pathog.*, vol. 12, no. 8, p. e1005704, Aug. 2016.
[6] R. Na and M. Gijzen, “Escaping host immunity: New tricks for plant pathogens,” *PLoS Pathog.*, vol. 12, no. 7, p. e1005631, Jul. 2016.
[7] M. F. Seidl, D. E. Cook, and B. P. H. J. Thomma, “Chromatin biology impacts adaptive evolution of filamentous plant pathogens,” *PLoS Pathog.*, vol. 12, no. 11, p. e1005920, Nov. 2016.
[8] M. Kamal and T. A. Bablu, “Machine Learning Models for Predicting Click-through Rates on social media: Factors and Performance Analysis,” *IJAMCA*, vol. 12, no. 4, pp. 1–14, Apr. 2022.
[9] W. Colonna, B. Brehm-Stecher, K. Shetty, and A. Pometto 3rd, “Rapid screening of natural plant extracts with calcium diacetate for differential effects against foodborne pathogens and a probiotic bacterium,” *Foodborne Pathog. Dis.*, vol. 14, no. 12, pp. 719–727, Dec. 2017.
[10] M. Nicolaisen, J. S. West, R. Sapkota, G. G. M. Canning, C. Schoen, and A. F. Justesen, “Fungal communities including plant pathogens in near surface air are similar across northwestern Europe,” *Front. Microbiol.*, vol. 8, p. 1729, Sep. 2017.
[11] A. Morales-Cruz *et al.*, “Profiling grapevine trunk pathogens in planta: A case for community-targeted DNA metabarcoding,” *bioRxiv*, bioRxiv, 06-Sep-2018.
[12] Y. Tao, L. Chen, M. Pan, F. Zhu, and D. Zhu, “Tailored biosensors for drug screening, efficacy assessment, and toxicity evaluation,” *ACS Sens.*, vol. 6, no. 9, pp. 3146–3162, Sep. 2021.
[13] A. Nassar and M. Kamal, “Ethical Dilemmas in AI-Powered Decision-Making: A Deep Dive into Big Data-Driven Ethical Considerations,” *IJRAI*, vol. 11, no. 8, pp. 1–11, 2021.
[14] H. C. S. Fukushima *et al.*, “Zebrafish toxicological screening could aid Leishmaniosis drug discovery,” *Lab. Anim. Res.*, vol. 37, no. 1, p. 27, Sep. 2021.
[15] J. N. Saldanha, A. Parashar, S. Pandey, and J. A. Powell-Coffman, “Multiparameter behavioral analyses provide insights to mechanisms of cyanide resistance in Caenorhabditis elegans,” *toxicological sciences*, vol. 135, no. 1, pp. 156–168, 2013.
[16] A. Nassar and M. Kamal, “Machine Learning and Big Data Analytics for Cybersecurity Threat Detection: A Holistic Review of Techniques and Case Studies,” *Intelligence and Machine Learning …*, 2021.
[17] F. Bouchama and M. Kamal, “Enhancing Cyber Threat Detection through Machine Learning-Based Behavioral Modeling of Network Traffic Patterns,” *IJBIBDA*, vol. 4, no. 9, pp. 1–9, Sep. 2021.
[18] B. Chen, A. Parashar, and S. Pandey, “Folded floating-gate CMOS biosensor for the detection of charged biochemical molecules,” *IEEE Sensors Journal*, vol. 11, no. 11, pp. 2906–2910, 2011.
[19] K. Usui *et al.*, “An ultra-rapid drug screening method for acetaminophen in blood serum based on probe electrospray ionization-tandem mass spectrometry,” *J. Food Drug Anal.*, vol. 27, no. 3, pp. 786–792, Jul. 2019.
[20] C. M. Legner, G. L. Tylka, and S. Pandey, “Robotic agricultural instrument for automated extraction of nematode cysts and eggs from soil to improve integrated pest management,” *Scientific Reports*, vol. 11, no. 1, p. 3212, 2021.
[21] S. H. Au, M. D. Chamberlain, S. Mahesh, M. V. Sefton, and A. R. Wheeler, “Hepatic organoids for microfluidic drug screening,” *Lab Chip*, vol. 14, no. 17, pp. 3290–3299, Sep. 2014.
[22] M. Zhu, “A review on recent robotic and analytic technologies in high throughput screening and synthesis for drug discovery,” *Lett. Drug Des. Discov.*, vol. 12, no. 9, pp. 778–784, Sep. 2015.
[23] T. Kong, N. Backes, U. Kalwa, C. Legner, G. J. Phillips, and S. Pandey, “Adhesive tape microfluidics with an autofocusing module that incorporates CRISPR interference:

applications to long-term bacterial antibiotic studies," *ACS sensors*, vol. 4, no. 10, pp. 2638–2645, 2019.

[24] Y.-D. Park *et al.*, "Identification of multiple cryptococcal fungicidal drug targets by combined gene dosing and drug affinity responsive target stability screening," *MBio*, vol. 7, no. 4, Sep. 2016.

[25] U. Kalwa, C. Legner, E. Wlezien, G. Tylka, and S. Pandey, "New methods of removing debris and high-throughput counting of cyst nematode eggs extracted from field soil," *PLoS One*, vol. 14, no. 10, p. e0223386, 2019.

[26] S. Laukkanen *et al.*, "In silico and preclinical drug screening identifies dasatinib as a targeted therapy for T-ALL," *Blood Cancer J.*, vol. 7, no. 9, pp. e604–e604, Sep. 2017.

[27] P. Pellegrini *et al.*, "A drug screening assay on cancer cells chronically adapted to acidosis," *Cancer Cell Int.*, vol. 18, no. 1, p. 147, Sep. 2018.

[28] Z. Njus *et al.*, "Flexible and disposable paper-and plastic-based gel micropads for nematode handling, imaging, and chemical testing," *APL bioengineering*, vol. 1, no. 1, 2017.

[29] J. P. Hilton, T. H. Nguyen, R. Pei, M. Stojanovic, and Q. Lin, "A microfluidic affinity sensor for the detection of cocaine," *Sens. Actuators A Phys.*, vol. 166, no. 2, pp. 241–246, Apr. 2011.

[30] A. Q. Beeman, Z. L. Njus, S. Pandey, and G. L. Tylka, "Chip technologies for screening chemical and biological agents against plant-parasitic nematodes," *Phytopathology*, vol. 106, no. 12, pp. 1563–1571, 2016.

[31] Y.-Y. He *et al.*, "Microfluidic-assisted assembly of fluorescent self-healing gel particles toward dual-signal sensors," *J. Mater. Sci.*, vol. 56, no. 26, pp. 14832–14843, Sep. 2021.

[32] M. Gil, P. Velez, F. Aznar-Ballesta, A. Mesegar-Ruiz, J. Munoz-Enano, and F. Martin, "Differential microfluidic sensors based on electroinductive-wave (EIW) transmission lines," in *2020 Fourteenth International Congress on Artificial Materials for Novel Wave Phenomena (Metamaterials)*, New York City, NY, USA, 2020.

[33] H. Landari, M.-A. Dussault, J. Ruel, A. Begin-Drolet, and A. Miled, "Biocompatible compact micropump with integrated unidirectional microvalves for low pressure microfluidic applications," *Sens. Actuators A Phys.*, vol. 276, pp. 246–258, Jun. 2018.

[34] B. Coelho *et al.*, "Digital microfluidics for nucleic acid amplification," *Sensors (Basel)*, vol. 17, no. 7, p. 1495, Jun. 2017.

[35] S. G. Yoon, B. J. Park, and S. T. Chang, "Highly sensitive microfluidic strain sensors with low hysteresis using a binary mixture of ionic liquid and ethylene glycol," *Sens. Actuators A Phys.*, vol. 254, pp. 1–8, Feb. 2017.

[36] X. Ding, Z. Njus, T. Kong, W. Su, C.-M. Ho, and S. Pandey, "Effective drug combination for Caenorhabditis elegans nematodes discovered by output-driven feedback system control technique," *Science advances*, vol. 3, no. 10, p. eaao1254, 2017.

[37] E. V. Moiseeva, A. A. Fletcher, and C. K. Harnett, "Thin-film electrode based droplet detection for microfluidic systems," *Sens. Actuators B Chem.*, vol. 155, no. 1, pp. 408–414, Jul. 2011.

[38] T. Kong, R. Brien, Z. Njus, U. Kalwa, and S. Pandey, "Motorized actuation system to perform droplet operations on printed plastic sheets," *Lab on a Chip*, vol. 16, no. 10, pp. 1861–1872, 2016.

[39] T. T. Vu, M. Fouet, A.-M. Gue, and J. Sudor, "A new and easy surface functionalization technnology for monitoring wettability in heterogeneous nano- and microfluidic devices," *Sens. Actuators B Chem.*, vol. 196, pp. 64–70, Jun. 2014.

[40] T.-K. Chiu, K.-F. Lei, C.-H. Hsieh, H.-B. Hsiao, H.-M. Wang, and M.-H. Wu, "Development of a microfluidic-based optical sensing device for label-free detection of circulating tumor cells (CTCs) through their lactic acid metabolism," *Sensors (Basel)*, vol. 15, no. 3, pp. 6789–6806, Mar. 2015.

[41] J. A. Carr, R. Lycke, A. Parashar, and S. Pandey, "Unidirectional, electrotactic-response valve for Caenorhabditis elegans in microfluidic devices," *Applied Physics Letters*, vol. 98, no. 14, 2011.