

PAPER • OPEN ACCESS

Biosensors for animal health and meat safety monitoring: farm-to-slaughterhouse continuum

To cite this article: Ivan Nastasijevic *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012063

View the [article online](#) for updates and enhancements.

You may also like

- [Innovated Conceptual Design of Loading Unloading Tool for Livestock at the Port](#)
Achmad Mustakim and Firmanto Hadi
- [Perspectives in meat processing](#)
D Vasilev, S Stajkovic, N Karabasil *et al.*
- [Attitudes and beliefs of consumers towards pig welfare and pork quality](#)
M Font-i-Furnols, M Skrlep and M Aluwé



IOP Publishing
**ENVIRONMENTAL
RESEARCH
2021**
A VIRTUAL CONFERENCE
15-19 NOVEMBER

FREE TO
ATTEND

REGISTER
NOW

Biosensors for animal health and meat safety monitoring: farm-to-slaughterhouse continuum

Ivan Nastasijevic¹, Radmila Mitrovic¹ and Sasa Jankovic¹

¹ Institute of Meat Hygiene and Technology, Kacanskog 13, Belgrade, Republic of Serbia

E-mail: ivan.nastasijevic@inmes.rs

Abstract. The meat supply chain needs to be managed for sufficient levels of consumer protection. Healthy animals are an essential precondition for a safe food supply, since zoonotic diseases, including meatborne pathogens, are a major threat to public health. Information about the livestock's general health, animal welfare and prevalence of major meatborne hazards such as *Salmonella*, *Campylobacter*, STEC and *Listeria monocytogenes* is of utmost importance for effective biosecurity control on farm. Early detection of these hazards in faecal samples, monitoring blood levels of metabolites relevant for animal welfare (hormones) and animal health (acute phase proteins) can provide high-level control in the animal farming industry. Multiplex biosensors for pathogens and metabolites in the farm-to-slaughterhouse continuum constitute a practical and cost-efficient tool for early detection of signs related to meat safety. Point-of-care multiplex biosensors are an advantage versus commonly used methods ELISA and RT-PCR, since they provide possibilities for early detection and do not require expensive equipment, trained personnel or significant time for sample transfer and analyses. Biosensors can improve meat inspection and meat safety controls, and can serve as a primary tool for monitoring food safety parameters and contribute to the modernization of veterinary inspection and risk-based meat safety assurance system.

1. Introduction

Meat safety is always at the forefront of public health and social-economic concerns [1]. Major meat safety challenges are associated with hazards that can be considered as traditional, new or emerging. This involves increased virulence and/or low infectious dose with antimicrobial resistance or resistance to other food-related stresses [1]. These hazards enter the meat chain in multiple points along the farm-slaughterhouse continuum. On the other hand, current, traditional meat inspection protocols (ante-mortem and post-mortem), based on visual inspection, palpation and incision, were not changed since the end of the nineteenth century, and were not fully efficient in terms of the current needs for consumer protection [2,3,4,5], since these protocols are intended for detection of traditional hazards (e.g. *Trichinella* spp., *Brucella* spp., *Mycobacterium bovis*, *Bacillus anthracis* and *Taenia solium/bovis* - cysticercosis) and can even increase cross-contamination due to palpation and/or incision procedures. The emerging hazards affecting safety of raw meat and poultry are bacterial pathogens such as Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 and non-O157 [6], *Salmonella*, e.g. the big five: *S. Typhimurium*, *S. Enteritidis*, *S. Infantis*, *S. Virchow*, *S. Hadar* [7], *Campylobacter jejuni*, *Yersinia enterocolitica* and *Toxoplasma gondii*, while *Listeria monocytogenes* remains a concern in ready-to-eat (RTE) processed meat products [1,8]. These hazards cannot be detected by traditional meat inspection methods, and therefore, there is a growing need for development of on-site, user-friendly and rapid



Content from this work may be used under the terms of the [Creative Commons Attribution 3.0 licence](https://creativecommons.org/licenses/by/3.0/). Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.

testing and pathogen detection methodologies in the farm-to-slaughterhouse continuum and with sufficient sensitivity and specificity, as are biosensors as point-of-care (PoC) devices. These biosensors are devices that have the potential to detect and quantify physiological, immunological and behavioural responses of livestock and multiple animal species [9]. PoC solutions are an advantage in comparison to the commonly used methods ELISA and RT-PCR, as well as other sensors available on the market that do not provide possibilities of early detection and require expensive equipment, trained personnel and significant time for sample transfer and analyses.

Novel biosensing methodologies offer highly specialised monitoring devices for the specific measurement of individual and/or multiple parameters covering an animal's physiology as well as monitoring an animal's environment. In addition to that, information on animal welfare and general animal health status are valuable to supplement the implementation of harmonized epidemiological indicators (HEI) and food chain information (FCI) flow, from farm to slaughterhouse (bottom-top) and backwards, from slaughterhouse to farm (top-down), setting up the foundation for effective implementation of risk-based meat safety assurance system (RB-MSAS).

2. Biosensor application for animal health and meat safety control: current status

Biosensors in livestock farm management provide significant benefits and applications in animal health and welfare monitoring, including detection of reproductive cycles [9, 10]. With the development of integrated systems and the internet of things (IoT), the continuously monitoring sensing devices are expected to become affordable. The data generated from integrated livestock monitoring should assist farmers to improve animal productivity. A biosensor is a device that recognizes a target biomarker characteristic for a particular pathogen and/or animal welfare or animal health molecules (indicators), via an immobilized sensing element called a bioreceptor (monoclonal antibody, RNA, DNA, aptamer, glycan, lectin, enzyme, tissue, whole cell) (Figure 1). The bioreceptor is an essential component as its biochemical characteristics assure high sensitivity and specificity of the biomarker detection and allow avoidance of interferences with other microorganisms or molecules present in the tested sample [11, 12]. It is challenging to provide high levels of sensitivity and specificity of biosensors for quantitative detection of biomarkers (pathogen, animal welfare & animal health indicator) in complex media such as are the matrixes collected from the production environment on farm and at slaughterhouse (e.g. faeces, saliva, blood, serum). Therefore, there is a need for PoC and/or automatic reliable detection and quantification tools that can foresee when disease is likely to occur before that any clinical sign appears in animals [11].

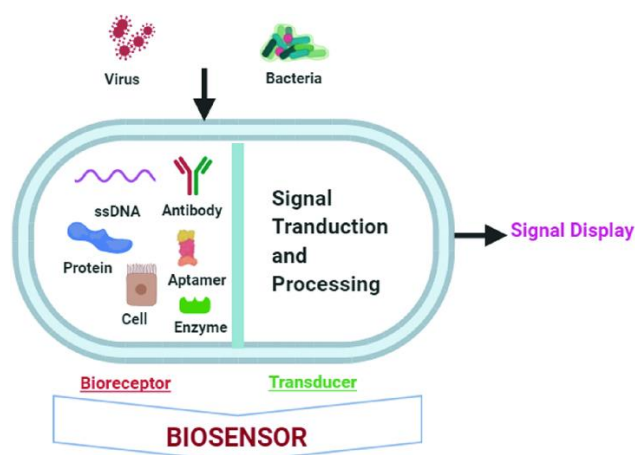


Figure 1. Biosensor mode of action [12]

2.1. Biosensors on farm

Biosensors can provide accurate and real-time detection for a wide range of conditions related to animal health and welfare on farm [9], such as: lameness in solipeds from acceleration data provided by ear tags, automatic 3D vision locomotion monitoring for cows, monitoring of physiological and behavioural stress in animals, monitoring of vocalisation sounds to assess response of broilers to environmental variables, cattle and pig cough monitoring as indicator of respiratory disease and environmental conditions, drinking behaviour of animals, automatic detection of health (body temperature) with a video-based infrared thermography camera, evaluating hormone profiles to improve automated oestrus detection [9] and faecal shedding of food borne pathogens [10].

2.1.1. Sensor for detection of metabolites

Mechanical sensors (pressure sensors). Designed to be used specifically in pastures and stables. The noseband and an electronic interface are connected to record, analyse and store data at 20 Hz at computer [13]. For example, the jaw movement is identified as a pressure peak through the transmission of the movement to the halter and the change in the tube pressure. The software can identify bites and/or chews [13].

Acoustic sensors. Used for the analysis of the jaw movement and grazing behaviour, precisely identifying chewing and biting to enable the estimate of the food intake in cattle [14].

Acceleration sensors. Used for monitoring of jaw movement and feeding behaviour. The static acceleration due to gravity and dynamic acceleration due to animal movement are measured [15].

Breath analyses biosensors. Enable non-invasive method used for disease diagnostics by detection and characterization of volatile organic compounds (VOCs) [9]. VOCs can be found in breath, blood, faeces, skin, urine and vaginal fluids of animals and humans [9]. Breath metabolites encompass gasses, (e.g. hydrogen and methane) and fatty acids, all of which can be used as specific biomarkers for detection of metabolic and pathologic processes. For example, a high level of glucose in blood is detected by presence of specific VOCs, e.g. ketones, ethanol and methanol [16]. In livestock, these biosensors can accurately identify bovine respiratory diseases (BRD) [17], brucellosis [18], bovine tuberculosis [19], Johne's diseases [20], ketoacidosis [21] and even foot and mouth (FMD) disease [22].

Perspiration metabolite biosensors. They have been developed mostly for human health monitoring, such as analysis of sweat for sodium concentration and lactate levels [23]. Such sensors can be also adapted to be used for animal welfare control, e.g. physical stress.

Tear fluid biosensors. The level of certain metabolites in tears can provide information about the concentration of these metabolites in blood. For example, a glucose sensor has been developed [24].

Progesterone analyses biosensor. This sensor was developed by integrating a selected aptamer specific for its binding properties with progesterone [9].

Salivary detection of metabolites. The metabolites detected in saliva can provide valuable information on animal welfare and disease. This is a non-invasive method where biomarkers in saliva are used instead of taking the blood samples. For example, a high level of uric acid in saliva could be connected with a metabolic syndrome, renal syndrome or physical stress, or salivary cortisol, which reflects the level of animal stress, can be monitored [25].

2.1.2. Sensors for detection of animal diseases

Bovine Respiratory Disease (BRD). This PoC biosensor is made to be sensitive and specific to anti-IgE present in commercial anti-BHV_1 (bovine Herpes Virus-1, the cause of BRD) and in real serum samples from cattle [26].

Bovine Viral Diarrhoea (BVD). The sensor can detect BVD antibodies in serum of cattle [27]. The detection time is 8 min, with detection limit of 10^3 CCID/ml in BVD samples.

Avian Influenza virus (AIV). The sensor is based on detection of immobilised H7N1 antibodies, providing low level of detection [28].

Foot and Mouth Diseases (FMD). The developed sensor includes a lateral flow immunochromatographic platform for the detection of antibodies against FMD proteins [29].

Mastitis. An indirect on-line sensor system based on the automated California mastitis test (CMT) in milk has been developed [30], or the recently developed sensor for detection of mastitis based on haptoglobin (Hp) [31].

Other. There are also other developed biosensors enabling PoC detection of ketosis and porcine reproductive and respiratory syndrome (PRRS) virus,

2.2. Biosensors in slaughterhouses

There is no wide commercial and routine use of biosensors in slaughterhouse for the purposes of meat safety monitoring, so far. On the other hand, several biosensors for detection of food(meat)borne pathogens are available. For example, lateral flow aptamer-based biosensors for PoC detection of *Salmonella* Enteritidis and *Escherichia coli* O157:H7 were developed with sensitivity level of 10^1 CFU/ml and 10 CFU/ml, respectively [32, 33]; DNA-based sensor for detection of *Campylobacter* in meat (poultry) samples with detection level of 1.5×10^1 CFU/g [34]; Cell-based sensors which have mammalian cells as sensing elements to detect the pathogens or toxins of *Clostridium perfringens* [35]; Antibody-based biosensors for detection of *Escherichia coli* [36], or; conductometric-based biosensors for *E. coli* at detection level from 1 to 10^3 CFU/mL [37]. However, the performance and detection limit of above mentioned biosensors were mainly tested with enriched bacterial suspension (in vitro) with scarcity of data when using a matrix from the production environment (e.g. straw, faeces, blood).

2.3. Biosensors in environmental control (slaughterhouse wastewater)

Slaughterhouses generate a substantial amount of wastewater, and the treatment protocols and post-treatment purity level of waste waters are regulated. Biochemical oxygen demand (BOD) is a widely used parameter to describe the level of organic pollution in water and wastewaters. Just recently, BOD biosensors were developed, based on detection of refractory compounds [38]. Namely, natural waters and slaughterhouse wastewaters have several specific refractory compounds that microorganisms are not able to use and degrade within the biosensor's short measuring time. A semi-specific biosensor is manufactured that uses *Aeromonas hydrophila* P69.1 to estimate BOD in high fat and grease content wastewaters, while a universal biosensor is manufactured that uses non-specific *Pseudomonas Fluorescens* P75. Service life of *A. hydrophila* and *P. Fluorescens* biosensors are 110 and 115 days, respectively. The measuring time is 20 minutes, and the biosensor based on *A. hydrophila* proved to be more accurate in measuring the fat content of the meat industry wastewaters [39].

3. Need for development of biosensors for use in the farm-slaughterhouse continuum (F2SC)

For the purposes of meat safety monitoring and control in the meat supply chain continuum, there is a need for development and optimization of multiplex biosensors which will be effectively used as PoC devices in F2SC [10]. Namely, these biosensors should preferably detect and quantify several key target molecules relevant for farm biosecurity (e.g. selected pathogens), animal welfare (e.g. selected hormones) and general animal health (e.g. acute phase proteins) and, thus, serve as excellent food safety management tools to improve the consumer protection (Figure 2). The major challenges are related to the functionalization and increase of biosensors' sensitivity, together with optimization of sampling protocols to enable accurate detection of key molecules in matrixes available in production environments (farm, slaughterhouse).

Healthy animals are an essential precondition for a safe food supply, since zoonotic diseases are a major threat to public health. Concerning livestock, early information about the prevalence of major health hazards of bacterial origin is of utmost importance for effective control on the farm. Early detection of *Salmonella*, *Campylobacter*, STEC O157, *Listeria monocytogenes* etc. in faecal samples, as well as monitoring blood levels of metabolites relevant for animal welfare and animal health, can provide high-level control in the animal farming and meat industry. Integration of such multiplex biosensors for pathogens and metabolites in the F2SC is a practical and cost-efficient tool for early detection of signs that meat safety is jeopardized.

The availability of relevant information, together with HEI for cattle, pigs, poultry (as well as other meat producing animals) will contribute to the FCI flow from farm-to-slaughterhouse (bottom-up) and vice versa, from slaughterhouse-to-farm (top-down) and enable implementation of a RB-MSAS [4].

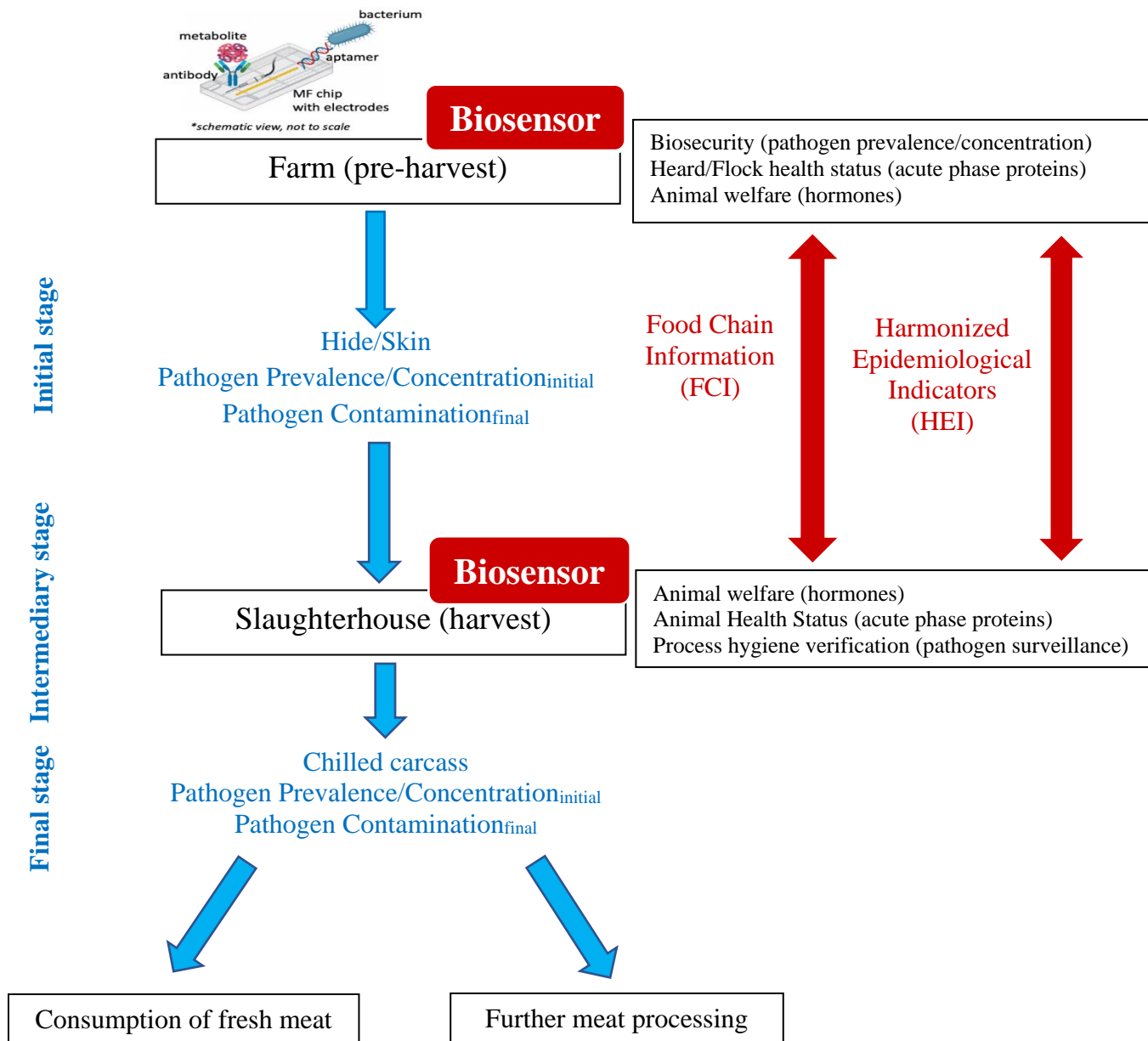


Figure 2. A model for practical implementation of multiplex, point-of-care biosensor in the farm-to-slaughterhouse continuum for animal health, animal welfare and meat safety monitoring

Acknowledgement

This study was supported by the Innovation Fund of the Republic of Serbia, Proof of Concept programme, according to the Contract (No. 161, dated 09.09.2020.) for the implementation of the project Development and Integration of Microfluidic Biosensors for Meat Safety Monitoring in Farm-to-Slaughterhouse Continuum` (ID5524) and by the Ministry of Education, Science and Technological

Development of the Republic of Serbia, according to the provisions of the Contract on research funding in 2021 (No. 451-03-9/2021-14/200050, dated 05.02.2021).

References

- [1] Sofos J 2008 Challenges to meat safety in the 21st century *Meat Sci.* **78** 3–13
- [2] Buncic S, Alban L and Blagojevic B 2019 From traditional meat inspection to development of meat safety assurance programs in pig abattoirs – The European situation *Food Control* **106** 106705
- [3] Nastasijevic I, Lakicevic B, Raseta M, Djordjevic V, Jankovic V, Mrdovic B and Brankovic-Lazic I 2018 Evaluation of pig welfare in lairage and process hygiene in a single abattoir. *Meat Tech.* **59** (1) 8–22
- [4] Nastasijevic I, Veskovc S and Milijasevic M 2020 Meat Safety: risk based assurance systems and novel technologies *Meat Tech.* **61** (2) 97–119
- [5] Blagojevic B, Nesbakken T, Alvseike O, Vågsholm I, Antic D, Johler S, Houf K, Meemken D, Nastasijevic I, Vieira Pinto M et al. L 2021 Drivers, opportunities, and challenges of the European risk-based meat safety assurance system. *Food Control* **124** 07870
- [6] EFSA 2020 Pathogenicity assessment of Shiga toxin-producing *Escherichia coli* (STEC) and the public health risk posed by contamination of food with STEC *EFSA J* **18** 5967
- [7] EFSA/ECDC 2019 The European Union One Health 2018 Zoonoses Report *EFSA J* **17** (12) 5926
- [8] Nastasijevic I, Milanov D, Velebit B, Djordjevic V, Swift C, Painset A and Lakicevic B. 2017 Tracking of *Listeria monocytogenes* in meat establishment using Whole Genome Sequencing as a food safety management tool: A proof of concept *Int. J. Food Microbiol.* **257** 157–64
- [9] Neethirajan S, Tuteja SK, Huang S-T and Kelton D 2017 Recent advancement in biosensors technology for animal and livestock health management *Biosens. Bioelectron* **98** 398–407
- [10] Nastasijevic I, Brankovic Lazic I and Petrovic Z 2019 Precision livestock farming in the context of meat safety assurance system The 60th International Meat Industry Conference MEATCON2019. *IOP Conf. Series: Earth and Environmental Science* **333** doi:10.1088/1755-1315/333/1/012014
- [11] Vidic J, Manzano M, Chang C-M and Jaffrezic-Renault, N 2017 Advanced biosensors for detection of pathogens related to livestock and poultry *Vet Res* **48** 11
- [12] Tewari A, Jain B, Brar B, Prasad G and Prasad M 2020 Biosensors: Modern Tools for Disease Diagnosis and Animal Health Monitoring *Biosensors in Agriculture: Recent Trends and Future Perspectives* Editors: Pudake R N Jain U, Kole C, Springer pp 387–414
- [13] Rutter S M 2000 Graze: a program to analyze recordings of the jaw movements of ruminants *Behav. Res. Methods Instrum. Comput* **32** (1) 86–92
- [14] Laca Wallis De Wries 2000 Acoustic measurement of intake and grazing behaviour of cattle. *Grass Forage Sci.* **55** (2) 97–104
- [15] Herinaina A I, Bindelle J, Mercatoris, B and Lebeau, F 2016 A review on the use of sensors to monitor cattle jaw movements and behaviour when grazing *Biotechnol. Agron. Soc. Environ.* **23** (S1) 273–86
- [16] Leopold J H, van Hooijdonk R T, Sterk P J, Abu-Hanna A., Schultz M J and Bos L D 2014 Glucose prediction by analyses of exhaled metabolites – a systematic review *BMS Anesthesiol* **14** 46
- [17] Burciaga-Robles L O, Holland B P, Step D I, Krehbiel C R, McMillen G I, Richards C J, Sims L E, Jeffers J D, Namjou K, McCann P J 2009 Evaluation of breath biomarkers and serum haptoglobin concentration for diagnosis of bovine respiratory disease in heifers newly arrived at a feedlot *Am. J. Vet. Res.* **70** (10) 1291–8
- [18] Knobloch H, Kohler H, Commander N, Reinhold P, Turner C, Chambers M, Pardo M and Sberveglieri G 2009 Volatile organic compounds (VOC) analysis for disease detection: proof of principle for field studies detecting paratuberculosis and brucellosis. *AIP Conf Proc* 195–7

- [19] Fend R, Geddes R, Lesellier S, Vordermeier H-M, Corner L A L, Gormley E, Costello E, Hewinson R G, Marlin D J, Woodman A C and Chambers M A 2005 Use of an electric nose to Diagnose *Mycobacterium bovis* infection in badgers and cattle *J. Clin. Microbiol.* **43** (4), 1745–51
- [20] Kumanan V, Nugen S R, Baeumner A J and Chang Y-F 2009 A biosensor assay for the detection of *Mycobacterium avium subsp. paratuberculosis* in fecal samples *J. Vet. Sci.* **10** (1) 35–42
- [21] Mottram T, Dobbelaar P, Schukken Y, Hobbs P and Bartlett P 1999 An experiment to determine the feasibility of automatically detecting hyperketonaemia in dairy cows *Livest. Prod. Sci.* **61** (1) 7–11
- [22] Christensen L S, Brehm K E, Skov J, Harlow K W, Christensen J and Haas B 2011 Detection of foot-and-mouth disease virus in the breath of infected cattle using a hand-held device to collect aerosols *J. Virol. Methods* **177** (1) 44–8
- [23] Schazmann B, Morris D, Slater C, Beirne S, Fay C, Reuveny R, Moyna N and Diamond D 2010 A wearable electrochemical sensor for the real-time measurement of sweat sodium concentration *Anal. Methods* **2** (4) 342–8
- [24] La Belle J T, Engelschall E, Lan K, Shah P, Saez N, Maxwell S, Adamson T, Abon-Eid M, McAferty K, Patel D R and Cook C B 2014 A disposable tear glucose biosensor-part 4: preliminary animal model study assessing efficacy, safety, and feasibility *J. Diabetes Sci. Technol.* **8** (1) 109–16
- [25] Yamaguchi M, Matsuda Y, Sasaki S, Sasaki M, Kadoma Y, Imai Y, Niwa D and Shetty V 2013 Immunosensor with fluid control mechanism for salivary cortisol analysis. *Biosens Bioelectron* **41** 186–91
- [26] Tarasov A, Gray D W, Tsai M Y, Shields N, Montrose A, Creedon N, Lovera P, O’Riordan A., Mooney M H and Vogel E M 2016 A potentiometric biosensor for rapid on-site disease diagnostics *Biosens Bioelectron* **79** 669–78
- [27] Montrose A, Creedon N, Sayers R, Barry S and O’Riordan A 2015 Novel single gold nanowire-based electrochemical immunosensor for rapid detection of bovine viral diarrhoea antibodies in Serum *J. Biosens. Bioelectron* **6** (3) 1–7
- [28] Wang R, Wang Y, Lassiter K, Li Y, Hargis B, Tung S, Berghman L and Bottje W 2009 Interdigitated array microelectrode based impedance immunosensor for detection of avian influenza virus H5N1 *Talanta* **79** (2) 159–64
- [29] Yang M, Caterer NR, Xu W and Goolia M 2015 Development of a multiplex lateral flow strip test for foot-and-mouth disease virus detection using monoclonal antibodies *J. Virol. Methods* **221** 119–26
- [30] Neitzel A-C, Stamer E, Junge W and Thaller G 2014 Calibration of an automated California Mastitis Test with focus on the device-dependent variation *Springer plus* **3** 760
- [31] Martins S A M, Martins V C, Cardoso F A, Germano J, Rodrigues M, Duarte C, Bexiga R, Cardoso S and Freitas P P 2019 Biosensors for On-Farm Diagnosis of Mastitis *Frontiers in Bioengineering and Biotechnology* **7** (186) 1–19
- [32] Fang Z, Wu W, Lu X and Zeng L 2014 Lateral flow biosensor for DNA extraction-free detection of *Salmonella* based on aptamer mediated strand displacement amplification. *Biosens Bioelectron* **56** 192–7
- [33] Wu W, Zhao S, Mao Y, Fang Z, Lu X and Zeng L. 2015 A sensitive lateral flow biosensor for *Escherichia coli* O157:H7 detection based on aptamer mediated strand displacement amplification *Anal Chim Acta.* **861** 62–8
- [34] Manzano M, Cecchini F, Fontanot M et al 2015 OLED-based DNA biochip for *Campylobacter* spp. detection in poultry meat samples *Biosens Bioelectron* **66** 271–6
- [35] Yoo S M and Lee S Y 2016 Optical biosensors for the detection of pathogenic microorganisms *Trends Biotechnol.* **34** 7–25
- [36] Jaffrezic-Renault N, Martelet C, Chevotot Y and Cloarec J P 2007 Biosensors and bio-bar code assays based on biofunctionalized magnetic microbeads *Sensors* **7** 589–614

- [37] El Ichi S, Leon F, Vossier L et al 2014 Microconductometric immunosensor for label-free and sensitive detection of gram-negative bacteria *Biosens Bioelectron* **54** 378–84
- [38] Chee G-J., Nomura Y, Ikebukuro K and Karube I 1999 Development of highly sensitive BOD sensor and its evaluation using preozonation *Anal Chim Acta* **394** 65–71
- [39] Raud M, Tenno T, Jogi E, Kikas T 2012 Comparative study of semi-specific *Aeromonas hydrophila* and universal *Pseudomonas fluorescens* biosensors for BOD measurements in meat industry wastewaters *Enzyme Microb. Technol.* **50** (4–5) 221–6