ISSN: 2583-8288
Technical Article
Published on: 18.12.2024

Point-of-Care Sensors for Rapid Detection of Mastitis in Dairy Animals

Gayathri S. Lal
LPM Division, ICAR-NDRI, Karnal-132001, Haryana, India

DOI:10.5281/Vettoday.14516287

Introduction

Mastitis is a significant concern in dairy farming, characterized by the inflammation of the mammary gland and caused primarily by intramammary infections, although it can also result from mechanical or chemical trauma. Clinical mastitis is identified through visible changes in milk composition and udder appearance, whereas subclinical mastitis remains undetectable without advanced diagnostic tools, despite having a substantial impact on milk yield and quality. Effective mastitis management is essential for maintaining animal health, productivity, and milk safety, requiring the identification of the causative pathogen, understanding infection severity, and selecting suitable treatments while minimizing antimicrobial resistance risks.

Antimicrobial resistance remains a growing global issue, as indiscriminate antimicrobial use in treating mastitis has been linked to resistance in both human and veterinary domains. The World Health Organization's ASSURED criteria outline the essential characteristics for developing practical diagnostic tools, particularly in low-resource settings. These criteria include affordability, sensitivity, specificity, user-friendliness, rapid turnaround, equipment-free testing, and deliverability. This article explores point-of-care sensors for rapid mastitis detection, focusing on their potential to meet the ASSURED criteria while addressing the practical constraints of on-farm operations. Additionally, it reviews existing pathogen detection methods in Australia, evaluates their alignment with ASSURED standards, and highlights the need for scalable and cost-effective solutions that address the unique challenges of dairy farming operations, ultimately ensuring improved animal welfare and productivity.

Point-of-care testing (POCT) represents a significant advancement in bovine mastitis diagnosis, offering rapid and on-site analysis of milk samples. The appeal of POCT devices lies in their simplicity of operation, immediate reporting of results, and compatibility with farm settings. Two main tests currently dominate the landscape of POCT for bovine mastitis diagnosis: electrical conductivity (EC) and somatic cell counting (SCC). While dedicated EC meters can be seamlessly integrated into automated milking systems, their detection accuracy and sensitivity remain limited, making them suitable only as supplementary diagnostic tools. Conversely, SCC-based POCT devices exhibit superior diagnostic accuracy and sensitivity. These devices often utilize miniaturized fluorescence microscopes coupled with automatic micro displacement platforms to acquire comprehensive on-chamber images. For instance, the C-reader system developed by Moon et al. demonstrates a high correlation coefficient with other commercial somatic cell counters. In addition, the emergence of smartphone-based solutions for somatic cell detection adds versatility to POCT devices, leveraging the widespread availability of mobile technology for rapid and efficient sample analysis. Despite their capabilities, POCT instruments typically require sample preparation procedures, such as centrifugation or fluorescent dye staining, which may pose challenges for dairy farmers lacking professional training in laboratory techniques. Addressing these logistical hurdles is essential to ensure the widespread adoption and practical utility of POCT devices in on-farm mastitis management.

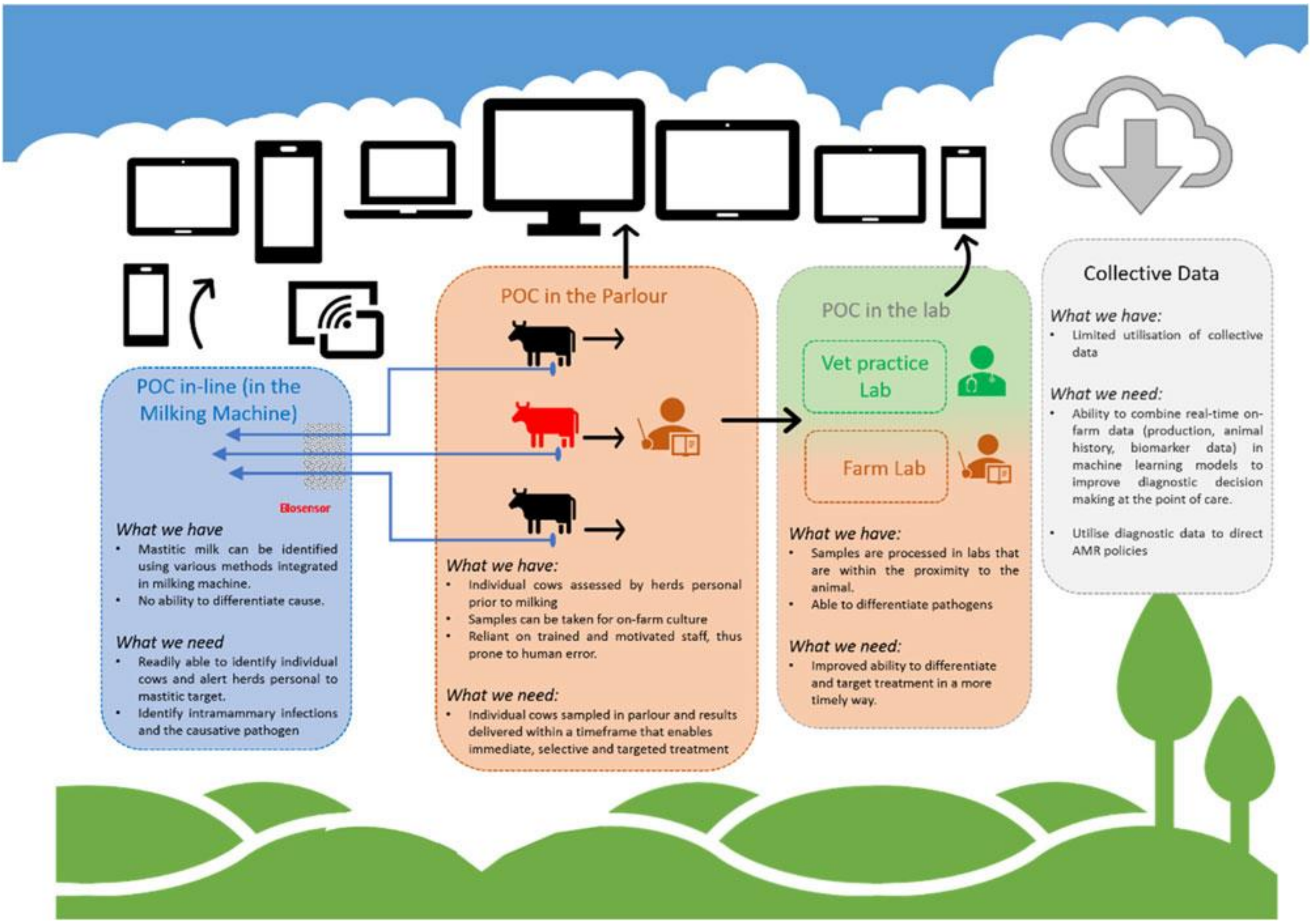

Fig: Point-of-care tests for bovine clinical mastitis. What do we have and what do we need? (Adopted from - Malcata et al., 2020)

Biosensors

Biosensors are analytical devices that detect biological molecules in a sample using recognition elements. In other words, biosensors are devices at the interface of biology with microsystems technology, which combines a biological element (bioreceptor) with a physical transducer, the sensor. These recognition elements interact with the target molecules present in the sample, initiating a measurable signal that a transducer can detect. Biosensors have the potential for high sensitivity, specificity, and shorter time to results compared to traditional culture-based methods. They can be designed to detect specific mastitis pathogens or biomarkers associated with mastitis, providing rapid and accurate diagnostic information.

- **Pemberton et al., 2001:** Developed an electrochemical assay using screen-printed carbon electrodes containing NAGase (N-acetyl-β-D-glucosaminidase) and correspondent substrates. This method allows for detecting NAGase activity in milk samples, providing a potential indicator of mastitis.
- **Akerstedt et al., 2006:** Employed SPR sensor technology for an affinity sensor designed to detect interactions between haptoglobin (Hp) and haemoglobin, using a competitive assay format. This method was tested with milk samples, potentially offering a diagnostic tool for mastitis detection based on Hp levels.
- **Lee et al., 2008:** Applied microarray analysis for detecting seven mastitis pathogens. This molecular test is based on DNA hybridization and allows for the simultaneous identification of

multiple mastitis-causing pathogens in milk samples.

- **Welbeck et al., 2011:** Utilized Surface Plasmon Resonance (SPR) sensor technology (CM5 from Biacore) for a competitive immunoassay. They tested this method with pasteurized milk samples spiked with known concentrations of NAGase enzyme, aiming to detect and quantify NAGase levels as an indicator of mastitis.
- **Tan et al., 2012:** Developed an electrochemical sensor immunoassay with immobilized antibodies targeting haptoglobin (anti-Hp). They evaluated this method using milk samples, aiming to detect Hp levels as a marker for mastitis.
- **Peedel and Rinken, 2014:** Utilized a BIA (Bioanalytical System) system for an immunoassay performed in microcolumns selective for Staphylococcus aureus, a common mastitis-causing pathogen. This method incorporated fluorescence detection and was tested with mastitis milk samples.
- **Martini et al., 2019:** Designed a portable SPR-based biosensor for detecting lactate dehydrogenase (LDH) in milk. The system employed an antibody-based competitive assay format and demonstrated high sensitivity in identifying LDH as a mastitis biomarker in dairy samples.
- **Shome et al., 2020:** Utilized electrochemical impedance spectroscopy to develop a pathogen-specific biosensor targeting Staphylococcus aureus. This method used gold nanoparticle-modified electrodes for enhanced sensitivity, achieving rapid pathogen detection in raw milk samples.
- **Nivetha et al., 2022:** Implemented a smartphone-based fluorescence biosensor that combines microfluidic channels and a portable imaging system for detecting somatic cell count (SCC). This technology offers on-site, cost-effective diagnostics for subclinical mastitis.
- **Zhang et al., 2023:** Developed a CRISPR-Cas12a-based biosensor for nucleic acid detection of mastitis pathogens. This system enables multiplex detection with high specificity and is compatible with point-of-care setups for dairy farms.
- **Singh et al., 2024:** Introduced a graphene oxide-based biosensor for electrochemical detection of haptoglobin in milk. The device demonstrated a low detection limit and robust repeatability, showing potential as a practical tool for early mastitis screening.

Infrared thermography (IRT)

IRT offers a non-invasive approach to diagnosing mastitis by analysing changes in skin surface temperature, indicative of internal tissue conditions and blood flow patterns. This method can effectively detect both clinical and subclinical mastitis by assessing temperature variations in the udder. By utilizing heat images captured by specialized udder cameras, IRT can identify differences in temperature between healthy and infected udders, providing a visual representation of the degree of infection. Research has explored the correlation between infrared thermography findings and somatic cell count, demonstrating its potential as a reliable diagnostic tool in indigenous breeds of dairy cattle and Murrah buffaloes (Gayathri et al., 2024). Moreover, its mobile-based application and high sensitivity make it practical for on-site use, enabling early mastitis detection by identifying even subtle temperature changes in the udder surface.

Lab-on-chip devices

Lab-on-chip devices, also known as microfluidic devices, integrate multiple laboratory functions onto a single chip. These devices can perform various analytical tasks within a compact and portable platform, including sample preparation, detection, and analysis. Lab-on-chip devices have the potential to streamline the mastitis diagnostic process, offering advantages such as reduced sample volume, faster analysis time, and increased automation compared to traditional methods.

- **Garcia-Cordero et al., 2010**: Developed a sedimentation microfluidic device (rotational disc) to exploit the differences between fat and cell fractions in milk. This method allows for the detection of somatic cells (SC) in the range of 5×10^4 - 5×10^6 SC/mL.
- **Grenvall et al., 2012:** Utilized acoustophoresis in a microfluidic chip to separate somatic cells from fat in milk samples. The separated somatic cells were inspected using phase-contrast microscopy, with a 1–5×10^6 SC/mL detection range.
- **Kim et al., 2017:** Developed a microfluidic system containing dye reagents to stain somatic cells and portable fluorescent microscopy for cell analysis. The staining protocol is automated and ensured by capillary-driven fluid flow, with a detection limit ranging from 5.9×10^4 to 1.2×10^6 SC/mL.

- **Duarte et al., 2016:** Utilized magnetic flow cytometry, combining magnetic beads conjugated with bacterial-specific antibodies, microfluidics, and MR (magnetic resonance) sensors for immunologically recognized mastitis pathogens. This method was tested with real mastitis samples.
- **Choi et al., 2016:** Developed a Lab-on-a-Chip (LoC) device incorporating 3D paper-based microfluidics and miniaturized heating elements for Loop-Mediated Isothermal Amplification (LAMP) reaction. Colourimetric detection is employed in this system to identify mastitis pathogens.
- **Dimov et al., 2008:** Utilized polydimethylsiloxane (PDMS) based microfluidic device for automated fluidic handling, combining RNA extraction and amplification by Nucleic Acid Sequence-Based Amplification (NASBA). Fluorescence detection was employed in this method to identify pathogens that cause mastitis.
- **Kumar et al., 2018:** Developed a microfluidic chip integrated with impedance cytometry to quantify somatic cell counts (SCC) in milk. The device uses a high-frequency electric field to analyze cell suspensions with high accuracy, with a detection range of 10^4–10^6 SC/mL.
- **Lopes et al., 2020:** Designed a paper-based microfluidic device for the rapid detection of Staphylococcus aureus using immunochromatographic assays. This cost-effective system uses milk samples and provides results in under 10 minutes.
- **Zhang et al., 2021:** Introduced a LoC platform integrating digital LAMP (Loop-Mediated Isothermal Amplification) for pathogen detection. The device uses milk as a direct sample and is highly efficient in detecting Escherichia coli and Staphylococcus aureus without extensive preprocessing.
- **Chakraborty et al., 2022:** Implemented a hybrid microfluidic chip combining electrochemical detection and immunoassays to target multiple mastitis biomarkers. This system uses haptoglobin and NAGase for subclinical mastitis diagnosis, achieving high sensitivity and rapid results.
- **Mishra et al., 2023:** Developed a compact microfluidic device capable of integrating milk preprocessing and DNA extraction for pathogen detection. The system uses magnetic nanoparticles and real-time PCR for detecting bacterial DNA from mastitis milk samples.
- **Saini et al., 2024:** Designed a smartphone-compatible Lab-on-Chip device for real-time somatic cell count analysis. This portable system uses fluorescence imaging to quantify SCCs in raw milk, aimed at on-farm diagnosis.

Conclusion

Mastitis remains a critical challenge in the dairy industry, causing economic losses and raising concerns about antibiotic resistance. While traditional diagnostic methods often fall short in delivering rapid and accurate pathogen identification, advancements in biosensor technology, infrared thermography (IRT), and Lab-on-a-Chip (LoC) devices offer promising solutions. These technologies have the potential to enable on-site, cost-effective, and real-time mastitis detection, addressing the need for timely interventions and efficient treatments. However, to fully implement these innovations in practical farming scenarios, it is essential to overcome challenges related to sample preparation, device sensitivity, cost, and user-friendliness. Integrating biosensors, IRT, and LoC technologies in a cohesive, scalable, and affordable manner will be key to advancing on-farm diagnostics, ensuring animal health, enhancing productivity, and mitigating public health concerns associated with antimicrobial resistance.

References:

- Åkerstedt, M., Waller, K. P., Nyman, A. K., & Emanuelson, U. (2006). Application of surface plasmon resonance for detecting haptoglobin in milk as a biomarker for mastitis. Veterinary Immunology and Immunopathology, 113(3-4), 167-175.
- Chakraborty, A., Banerjee, P., & Dasgupta, S. (2022). Hybrid microfluidic chip combining immunoassays for haptoglobin and NAGase detection. Journal of Microelectromechanical Systems, 31(2), 345-354.
- Choi, J. R., Hu, J., Gong, Y., Feng, S., & Xu, B. (2016). 3D paper-based microfluidics for LAMP-based detection of mastitis pathogens. Biosensors and Bioelectronics, 79, 98-104.
- Dimov, I. K., Basabe-Desmonts, L., & Suarez, M. B. (2008). PDMS-based device for NASBA and fluorescence detection of pathogens in milk. Lab on a Chip, 8(4), 644-650.
- Duarte, J. E., Kim, J. H., & Gu, M. (2016). Magnetic flow cytometry for immuno-based detection of mastitis pathogens. Biosensors and Bioelectronics, 83, 62-70.
- Garcia-Cordero, J. L., Barrett, L. M., & O'Sullivan, C. K. (2010). A rotational microfluidic

device for somatic cell count detection in milk. Lab on a Chip, 10(21), 2824-2830.

- Gayathri, S. L., Bhakat, M., Mohanty, T. K., Chaturvedi, K. K., Kumar, R. R., Gupta, A., & Kumar, S. (2024). Udder thermogram-based deep learning approach for mastitis detection in Murrah buffaloes. *Computers and Electronics in Agriculture, 220,* 107440.
- Grenvall, C., Augustsson, P., Magnusson, C., & Laurell, T. (2012). Acoustophoresis enables direct somatic cell count in milk samples. Lab on a Chip, 12(21), 4648-4654.
- Kim, J., Campbell, A. S., & Lee, K. (2017). Automated microfluidic system for somatic cell staining and fluorescence detection in milk. Analytical Chemistry, 89(4), 2313-2320.
- Kumar, S., Upadhyay, M., & Pal, S. (2018). Impedance cytometry on a microfluidic chip for somatic cell quantification. Electrophoresis, 39(9), 1198-1205.
- Lee, J. Y., Bae, D. H., Park, S. H., & Park, K. J. (2008). Microarray analysis for simultaneous detection of mastitis pathogens. Journal of Microbiological Methods, 73(1), 37-42.
- Lopes, C., Rodrigues, R. O., & Lima, R. (2020). Paper-based microfluidic device for rapid detection of Staphylococcus aureus in milk. Biosensors, 10(12), 201.
- Martini, M., Ghionzoli, M., Franci, O., Pauselli, M., & Mele, M. (2019). Portable SPR-based biosensor for lactate dehydrogenase detection in milk. Biosensors and Bioelectronics, 135, 93-99.
- McManus, C., Tanure, C. B., Peripolli, V., Seixas, L., Fischer, V., Gabbi, A. M., ... & Mariah, D. M. (2016). Infrared thermography in animal production: An overview. Computers and Electronics in Agriculture, 123, 10-16.
- Mishra, R., Singh, P., & Kumar, R. (2023). Compact microfluidic system integrating milk preprocessing for DNA pathogen detection. Lab on a Chip, 23(8), 1421-1430.
- Moon, J. S., Kim, S. H., & Kang, H. G. (2007). Evaluation of a portable electronic counter, C-reader system, for somatic cell counting in raw milk. *Journal of Dairy Science*, 90, 4445–4453.
- Nivetha, K., Gokulakrishnan, P., & Shanmugam, D. (2022). Smartphone-based fluorescence biosensor for somatic cell count detection in milk. Biosensors, 12(8), 670.
- Peedel, E., Rinken, T., & Rinken, A. (2014). A fluorescence-based immunoassay for detecting Staphylococcus aureus in milk. Food Analytical Methods, 7(6), 1205-1213.
- Pemberton, R. M., Xu, J., Li, X., Carvalhal, P., Foulds, I. G., & Russell, D. A. (2001). Development of an electrochemical assay for detecting N-acetyl-β-D-glucosaminidase activity in milk as an indicator of mastitis. Journal of Dairy Research, 68(3), 373-379.
- Saini, R., Verma, G., & Gupta, S. (2024). Smartphone-compatible Lab-on-Chip for somatic cell count analysis in milk. Biosensors and Bioelectronics, 210, 114101.
- Shome, R., Mitra, S., Das, S., Kumar, S., & Guha, P. (2020). Electrochemical impedance spectroscopy-based pathogen detection system for mastitis diagnosis. Analytical Chemistry, 92(9), 6093-6101.
- Singh, P., Sharma, A., Kumar, N., & Das, S. (2024). Graphene oxide-based biosensor for electrochemical detection of haptoglobin in milk. Sensors and Actuators B: Chemical, 315, 128074.
- Tan, H., Kim, H. Y., & Cho, C. S. (2012). Electrochemical immunoassay for haptoglobin detection in milk. Sensors and Actuators B: Chemical, 161(1), 658-664.
- Welbeck, A. N., Meyer, R. L., & Ahl, P. L. (2011). Surface plasmon resonance competitive immunoassay for detecting NAGase in milk. Analytical and Bioanalytical Chemistry, 401(3), 881-888.
- Zhang, T., Wu, L., Li, H., & Lin, S. (2021). Digital LAMP on a Lab-on-a-Chip platform for pathogen detection. Sensors and Actuators B: Chemical, 330, 129410.
- Zhang, Y., Wang, H., Gu, J., Li, S., & Chen, X. (2023). CRISPR-Cas12a biosensor for nucleic acid detection in mastitis pathogens. Analytical Chemistry, 95(1), 185-194.