

PCR-Based Diagnosis of Clostridial Enteric Infections in Sheep and Goats

Sudeep Solanki¹ & Kamal Purohit²,

¹Assistant Professor, Department of Microbiology, College of Veterinary and Animal Science, Udaipur, Rajasthan, India

³Assistant Professor, Department of Veterinary Pathology, College of Veterinary and Animal Science, Udaipur, Rajasthan, India

Email: ¹sudeepdrsolkanki@gmail.com,

drsudeepsolkanki@rediffmail.com

[DOI:10.5281/Vettoday.15734906](https://doi.org/10.5281/Vettoday.15734906)

Clostridial enteric infections represent a significant threat to the health and productivity of sheep and goat populations globally, resulting in substantial economic losses due to mortality, reduced growth rates, and decreased production efficiency ([Vidić et al., 2017](#)). Accurate and rapid diagnosis is paramount for effective disease management and control, enabling timely interventions and minimizing the impact of these infections on animal welfare and farm profitability. Traditional diagnostic methods, such as bacterial culture and histopathology, have limitations in terms of sensitivity, specificity, and turnaround time, which can hinder prompt and appropriate treatment decisions ([Farouk et al., 2021](#)). Molecular diagnostic techniques, including polymerase chain reaction, quantitative PCR (qPCR), and loop-mediated isothermal amplification, offer enhanced sensitivity and specificity for detecting Clostridial pathogens in clinical samples, enabling more accurate and timely diagnosis of enteric infections in sheep and goats ([Amjad, 2020](#)). These advanced molecular approaches overcome the constraints of conventional methods by directly targeting the genetic material of the causative agents, providing a more precise and rapid means of identification.

Polymerase chain reaction assays have emerged as a cornerstone in molecular diagnostics, offering unparalleled sensitivity and specificity for pathogen detection ([Dyussebayev et al., 2021](#)). Real-time quantitative PCR assays have demonstrated exceptional diagnostic capabilities, achieving sensitivity and specificity levels of 99% and 100%, respectively ([Nemati et al., 2023](#)). The ability to detect and differentiate multiple pathogens simultaneously through multiplex PCR assays further enhances diagnostic efficiency, particularly in cases of mixed infections, which are common in livestock populations ([Xu et al., 2019](#)). Isothermal amplification methods like loop-mediated isothermal amplification have emerged as valuable tools for rapid and point-of-

care diagnostics. These techniques offer the advantage of simple instrumentation and rapid turnaround times, making them well-suited for on-site detection of Clostridial pathogens in resource-limited settings ([Le & Vu, 2017](#)). Furthermore, advancements in immunological and nucleic acid-based techniques have revolutionized the detection of plant pathogens, inspiring applications in animal diagnostics, allowing for quick and reliable identification of disease-causing organisms ([Cahill, 1999](#)). Despite the remarkable progress in molecular diagnostics, the integration of these advanced techniques into routine diagnostic microbiology laboratories has been slower than anticipated, largely due to the

higher costs associated with reagents, equipment, and specialized training ([Vira et al., 2016](#)).

The effective implementation of molecular diagnostics necessitates careful consideration of several factors, including sample collection and processing, assay selection, data interpretation, and quality control. Optimal sample collection and processing methods are crucial for preserving the integrity of nucleic acids and minimizing the risk of false-negative results. Appropriate selection of molecular assays based on the target pathogen, clinical presentation, and desired turnaround time is essential for accurate diagnosis. Comprehensive data interpretation, considering both qualitative and quantitative results, is necessary for informed clinical decision-making. Moreover, rigorous quality control measures, including the use of positive and negative controls, are vital for ensuring the reliability and accuracy of molecular diagnostic testing. Molecular methods like polymerase chain reaction and probe-based tests offer high specificity and sensitivity but demand sterile environments and complex nucleic acid extraction ([Thaitrong et al., 2013](#)). Molecular diagnostic tests are invaluable because they can detect specific biological molecules and have become user-friendly. The transition of PCR from research to clinical laboratories in the early 1990s significantly improved clinical diagnostics by enhancing performance and reducing turnaround time ([Tsongalis & Silverman, 2006](#)).

The advent of molecular diagnostics has revolutionized the field of laboratory medicine, enabling the detection of individual biological molecules with unprecedented sensitivity and specificity ([Sokolenko & Imyanitov, 2018](#)). Molecular techniques, initially complex and accessible primarily to specialized scientists, have now become more widely adopted, transforming clinical medicine and improving patient outcomes ([Dadzie et al., 2010](#); [Siavashy et al., 2024](#)). Point-of-care nucleic acid testing is becoming increasingly prevalent in both developed and developing countries, particularly in situations demanding quick results and where centralized laboratory facilities are limited ([Niemz et al., 2011](#)). These advancements have enhanced the understanding of numerous diseases at the molecular level, contributing to the unraveling of the molecular pathology of common disorders. ([Lo et al., 2009](#); [Netto et al., 2003](#)). Molecular

diagnostic techniques, like next-generation sequencing and gene expression assays, have significantly advanced medical diagnostics. The rise of molecular diagnostics is pivotal in identifying genetic mutations, which is essential in customizing treatment strategies, particularly in cancer therapy ([Netto et al., 2003](#); [Sarnecka et al., 2019](#)). The development of user-friendly molecular analysis methods has greatly facilitated the practical applications of molecular oncology ([Sokolenko & Imyanitov, 2018](#)). As molecular diagnostic methods advance, their role in identifying disease markers and customizing treatments is set to grow, thereby improving patient care.

The expansion of molecular diagnostics and genetic testing is projected to continue in the coming years, driven by ongoing technological advancements, increasing demand for personalized medicine, and the growing recognition of the clinical utility of molecular information ([De et al., 2013](#)). Automation has significantly enhanced clinical chemistry and hematology laboratories, improving efficiency, reducing costs, and minimizing laboratory errors. Further innovations in genetics, genomics, and mass spectrometry are expected to facilitate a new era of precision medicine ([Wilson et al., 2022](#)). These advances will allow for the development of more sophisticated molecular assays, capable of detecting a wider range of pathogens, predicting disease risk, and tailoring treatment strategies to individual patients ([Malone et al., 2020](#)). The transition from single-gene investigations to comprehensive molecular network research promises faster and more efficient discovery of clinical implications. Molecular characterisation of tumor cells allows for refined cancer classifications, potentially guiding treatment decisions ([Rahman, 2015](#)). These molecular tests are indispensable for pinpointing disease markers and tailoring treatments to individual needs.

The implementation of molecular diagnostics in veterinary medicine offers considerable benefits, including more accurate and timely diagnoses of infectious diseases, improved disease surveillance and control, and the potential for personalized treatment strategies. Real-time PCR assays enable rapid and sensitive detection of pathogens, facilitating early intervention and preventing the spread of infection

within animal populations. The ability to detect genetic variations associated with disease susceptibility or drug response can inform breeding programs and treatment decisions. The application of molecular diagnostics in veterinary settings is particularly relevant for diseases that are difficult to diagnose using traditional methods, such as bacterial and viral infections with non-specific clinical signs. Furthermore, molecular techniques can be used to identify antimicrobial resistance genes, guiding the selection of appropriate antimicrobial and preventing the development of resistance.

Molecular diagnostics also offer promise for the development of novel therapeutic interventions, including targeted therapies and psychotherapies. Molecular genetics helps pinpoint genes linked to desirable traits, paving the way for precise breeding programs ([Hernández-Patlan et al., 2023](#)). Identifying genetic predispositions to disease enables proactive health management. Ultimately, the integration of molecular diagnostics into veterinary practice will improve animal health and welfare, enhance food safety, and protect public health. The development of molecular techniques has greatly aided in identifying disease markers and tailoring treatments to individual needs, signifying a shift towards more precise and effective healthcare strategies ([Chehab, 1993](#); [Ginsburg & Phillips, 2018](#)). The emergence of high-throughput technologies for altered cellular molecule detection has transformed cancer diagnostics ([Sethi et al., 2013](#)).

Molecular imaging plays a crucial role in differentiating between healthy and tumorous tissue, thereby enhancing surgical precision and outcomes ([Degrauwe et al., 2019](#)). This approach offers non-invasive tools for visualising biological processes at the cellular and molecular level, improving disease diagnosis, staging, and treatment ([Gomari et al., 2022](#)). This is particularly useful for diseases with genetic predispositions. The utilisation of advanced reproductive technologies for early animal selection, along with genomic technologies for predicting genetic merit, may significantly increase genetic gain rates ([Raadsma & Tammen, 2005](#)). Molecular biology techniques are useful in clinical practice for determining the underlying cause of disease and its treatment ([Senft &](#)

[LeVine, 2005](#)). The development of molecular imaging has facilitated the non-invasive visualisation of biological markers and potential therapeutic targets in both primary tumours and metastases.

References

- Amjad, M. (2020). An Overview of the Molecular Methods in the Diagnosis of Gastrointestinal Infectious Diseases [Review of An Overview of the Molecular Methods in the Diagnosis of Gastrointestinal Infectious Diseases]. *International Journal of Microbiology*, 2020, 1. Hindawi Publishing Corporation. <https://doi.org/10.1155/2020/8135724>
- Cahill, D. M. (1999). Detection, identification and disease diagnosis of soilborne pathogens. *Australasian Plant Pathology*, 28(1), 34. <https://doi.org/10.1071/ap99005>
- Chehab, F. (1993). Molecular Diagnostics: Past, present, and future [Review of Molecular Diagnostics: Past, present, and future]. *Human Mutation*, 2(5), 331. Wiley. <https://doi.org/10.1002/humu.1380020502>
- Dadzie, O. E., Neat, M., Emley, A., Bhawan, J., & Mahalingam, M. (2010). Molecular Diagnostics—An Emerging Frontier in Dermatopathology [Review of Molecular Diagnostics—An Emerging Frontier in Dermatopathology]. *American Journal of Dermatopathology*, 33(1), 1. Lippincott Williams & Wilkins. <https://doi.org/10.1097/dad.0b013e3181d7a2c0>
- De, J., Carlson, B., Caputo, N., Vojta, D., Sandy, L. G., & Stevens, S. (2013). Growth of Molecular Diagnostics and Genetic Testing in the USA, 2008–2011: Analysis and Implications. *Personalized Medicine*, 10(8), 785. <https://doi.org/10.2217/pme.13.84>
- Degrauwe, N., Hocquelet, A., Digkila, A., Schaefer, N., Denys, A., & Durán, R. (2019). Theranostics in Interventional Oncology: Versatile Carriers for Diagnosis and Targeted Image-Guided Minimally Invasive Procedures [Review of Theranostics in Interventional Oncology:

- Versatile Carriers for Diagnosis and Targeted Image-Guided Minimally Invasive Procedures]. *Frontiers in Pharmacology*, 10. *Frontiers Media*. <https://doi.org/10.3389/fphar.2019.00450>
- Dyussebayev, K., Sambasivam, P., Bar, I., Brownlie, J., Shiddiky, M. J. A., & Ford, R. (2021). Biosensor Technologies for Early Detection and Quantification of Plant Pathogens [Review of Biosensor Technologies for Early Detection and Quantification of Plant Pathogens]. *Frontiers in Chemistry*, 9. *Frontiers Media*. <https://doi.org/10.3389/fchem.2021.63624>
- 5
- Farouk, M. M., El-Molla, A., Salib, F. A., & Soliman, Y. A. (2021). Epidemiology of Salmonella Species in Diarrheic Sheep and Goats. *Pakistan Journal of Zoology*, 54(1). <https://doi.org/10.17582/journal.pjz/20201012161016>
- Ginsburg, G. S., & Phillips, K. A. (2018). Precision Medicine: From Science To Value [Review of Precision Medicine: From Science To Value]. *Health Affairs*, 37(5), 694. *Project HOPE*. <https://doi.org/10.1377/hlthaff.2017.1624>
- Gomari, M. M., Abkhiz, S., Pour, T. G., Lotfi, E., Rostami, N., Monfared, F. N., Ghobari, B., Mosavi, M., Alipour, B., & Dokholyan, N. V. (2022). Peptidomimetics in cancer targeting [Review of Peptidomimetics in cancer targeting]. *Molecular Medicine*, 28(1). *BioMed Central*. <https://doi.org/10.1186/s10020-022-00577-3>
- Hernández-Patlan, D., Téllez-Isaías, G., Hernández-Velasco, X., & Solís-Cruz, B. (2023). Editorial: Technological strategies to improve animal health and production. *Frontiers in Veterinary Science*, 10. <https://doi.org/10.3389/fvets.2023.120617>
- 0
- Le, D. T., & Vu, N. T. (2017). Progress of loop-mediated isothermal amplification technique in molecular diagnosis of plant diseases. *Applied Biological Chemistry*, 60(2), 169. <https://doi.org/10.1007/s13765-017-0267-y>
- Lo, Y. M. D., Wong, I. H. N., Tsui, N. B. Y., & Lam, C. (2009). Molecular Biological Analyses and Molecular Pathology in Clinical Chemistry. In *Encyclopedia of Analytical Chemistry*. <https://doi.org/10.1002/9780470027318.a0534.pub2>
- Malone, E. R., Oliva, M., Sabatini, P., Stockley, T., & Siu, L. L. (2020). Molecular profiling for precision cancer therapies [Review of Molecular profiling for precision cancer therapies]. *Genome Medicine*, 12(1). *BioMed Central*. <https://doi.org/10.1186/s13073-019-0703-1>
- Nemati, G., Romanò, A., Wahl, F., Berger, T., Rojo, L. V., & Graber, H. U. (2023). Bovine Staphylococcus aureus: a European study of contagiousness and antimicrobial resistance. *Frontiers in Veterinary Science*, 10. <https://doi.org/10.3389/fvets.2023.115455>
- 0
- Netto, G. J., Saad, R., & Dysert, P. A. (2003). Diagnostic Molecular Pathology: Current Techniques and Clinical Applications, Part I. *Baylor University Medical Center Proceedings*, 16(4), 379. <https://doi.org/10.1080/08998280.2003.11927931>
- Niemz, A., Ferguson, T. M., & Boyle, D. S. (2011). Point-of-care nucleic acid testing for infectious diseases [Review of Point-of-care nucleic acid testing for infectious diseases]. *Trends in Biotechnology*, 29(5), 240. *Elsevier BV*. <https://doi.org/10.1016/j.tibtech.2011.01.007>
- Raadsma, H. W., & Tammen, I. (2005). Biotechnologies and their potential impact on animal breeding and production: a review [Review of Biotechnologies and their potential impact on animal breeding and production: a review]. *Australian Journal of Experimental Agriculture*, 45(8), 1021. *CSIRO Publishing*. <https://doi.org/10.1071/ea05073>

- Rahman, M. M. (2015). Personalized Medicine in Cancer. *Journal of Bangladesh College of Physicians and Surgeons*, 32(3), 153. <https://doi.org/10.3329/jbcps.v32i3.26054>
- Sarnecka, A., Nawrat, D., Piwowar, M., Ligeza, J., Swadźba, J., & Wójcik, P. (2019). DNA extraction from FFPE tissue samples – a comparison of three procedures. *Współczesna Onkologia*, 23(1), 52. <https://doi.org/10.5114/wo.2019.83875>
- Senft, A. P., & LeVine, A. M. (2005). Basic molecular biology. *Paediatric Respiratory Reviews*, 6(3), 199. <https://doi.org/10.1016/j.prrv.2005.06.006>
- Sethi, S., Ali, S., Philip, P. A., & Sarkar, F. H. (2013). Clinical Advances in Molecular Biomarkers for Cancer Diagnosis and Therapy [Review of Clinical Advances in Molecular Biomarkers for Cancer Diagnosis and Therapy]. *International Journal of Molecular Sciences*, 14(7), 14771. Multidisciplinary Digital Publishing Institute. <https://doi.org/10.3390/ijms140714771>
- Siavashy, S., Soltani, M., Rahimi, S., Hosseinali, M., Guilandokht, Z., & Raahemifar, K. (2024). Recent advancements in microfluidic-based biosensors for detection of genes and proteins: Applications and techniques. *Biosensors and Bioelectronics X*, 19, 100489. <https://doi.org/10.1016/j.biosx.2024.100489>
- Sokolenko, A. P., & Imyanitov, E. N. (2018). Molecular Diagnostics in Clinical Oncology [Review of Molecular Diagnostics in Clinical Oncology]. *Frontiers in Molecular Biosciences*, 5. Frontiers Media. <https://doi.org/10.3389/fmolb.2018.00076>
- Thaitrong, N., Charlermroj, R., Himananto, O., Seepiban, C., & Karoonuthaisiri, N. (2013). Implementation of Microfluidic Sandwich ELISA for Superior Detection of Plant Pathogens. *PLoS ONE*, 8(12), 31. <https://doi.org/10.1371/journal.pone.0083231>
- Tsongalis, G. J., & Silverman, L. M. (2006). Molecular diagnostics: A historical perspective. *Clinica Chimica Acta*, 369(2), 188. <https://doi.org/10.1016/j.cca.2006.02.044>
- Vidić, J., Manzano, M., Chang, C.-M., & Jaffrézic-Renault, N. (2017). Advanced biosensors for detection of pathogens related to livestock and poultry [Review of Advanced biosensors for detection of pathogens related to livestock and poultry]. *Veterinary Research*, 48(1). BioMed Central. <https://doi.org/10.1186/s13567-017-0418-5>
- Vira, H. J., Bhat, V., & Chavan, P. (2016). Diagnostic Molecular Microbiology and its applications: Current and Future Perspectives. *Clinical Microbiology and Infectious Diseases*, 1(1). <https://doi.org/10.15761/cmids.1000105>
- Wilson, S., Steele, S., & Adeli, K. (2022). Innovative technological advancements in laboratory medicine: Predicting the lab of the future. *Biotechnology & Biotechnological Equipment*, 36. <https://doi.org/10.1080/13102818.2021.2011413>
- Xu, X., Yang, F., Zhāng, Q., Xu, Y., Huang, J., Fu, M., & Zhang, W. (2019). Development of a multiplex TaqMan qPCR assay for simultaneous detection and differentiation of four DNA and RNA viruses from clinical samples of sheep and goats. *Journal of Virological Methods*, 266, 58. <https://doi.org/10.1016/j.jviromet.2019.01.015>