

## Electrochemical Bioassay for Haptoglobin Quantification: Application in Bovine Mastitis Diagnosis

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**Abstract :** Mastitis is the most relevant inflammatory disease in cattle, affecting the animal health and causing important economic losses on dairy farms. This disease takes place in the mammary gland or udder when some opportunistic microorganisms, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, etc., invade the teat canal. According to the severity of the inflammation, mastitis can be classified as sub-clinical, clinical and chronic. Standard methods for mastitis detection include counts of somatic cells, cell culture, electrical conductivity of the milk, and California test (evaluation of “gel-like” matrix consistency after cell lysed with detergents). However, these assays present some limitations for accurate detection of subclinical mastitis. Currently, haptoglobin, an acute phase protein, has been proposed as novel and effective biomarker for mastitis detection. In this work, an electrochemical biosensor based on polydopamine-modified magnetic nanoparticles (MNPs@pDA) for haptoglobin detection is reported. Thus, MNPs@pDA has been synthesized by our group and functionalized with hemoglobin due to its high affinity to haptoglobin protein. The protein was labeled with specific antibodies modified with alkaline phosphatase enzyme for its electrochemical detection using an electroactive substrate (1-naphthyl phosphate) by differential pulse voltammetry. After the optimization of assay parameters, the haptoglobin determination was evaluated in milk. The strategy presented in this work shows a wide range of detection, achieving a limit of detection of 43 ng/mL. The accuracy of the strategy was determined by recovery assays, being of 84 and 94.5% for two Hp levels around the cut off value. Milk real samples were tested and the prediction capacity of the electrochemical biosensor was compared with a Haptoglobin commercial ELISA kit. The performance of the assay has demonstrated this strategy is an excellent and real alternative as screen method for sub-clinical bovine mastitis detection.

**Keywords :** bovine mastitis, haptoglobin, electrochemistry, magnetic nanoparticles, polydopamine

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