VII CONGRESO NACIONAL DE NANOTECNOLOGÍA
12 AL 15 DE NOVIEMBRE 2023
PUCÓN, CHILE

T602: Applied nanotechnology in industry, transfer, and safety oversight

Smartphone-based nano-genosensor for on-site detection of bovine tuberculosis using saliva as a diagnostic fluid

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Bovine tuberculosis is a zoonotic infectious disease caused by Mycobacterium bovis, which produces serious impacts in productivity and competitiviness of the dairy cattle sector. The intradermal tuberculin test is the standard method for rapid screening of M. bovis, which is based on a delayed hypersensitivity reaction. Despite its low cost and easiness of application, this test has a low response time (48-72 h) and suffers from diagnostic limitations that prevents from a better control and eradication of the disease [1,2].

The prototype developed herein consists of three subsystems: (i) the “turn on” nano-genosensor (NGS) based on fluorescent cadmium telluride nanocrystals (quantum dots) functionalized with a nucleotide probe that was bioinformatically designed to recognize DNA from a particular region of the Mycobacterium tuberculosis complex; (ii) a device (housing) with UV illumination and design features to optimize excitation/light emission from the NGS-containing microplates and (iii) a mobile phone application that digitally processes the optical signal emitted by the NGS (QDsReader software).The preanalytical stage involving the collection and preparation of cows' saliva for DNA purification was optimized for the processing of 96 bovine samples within 4.5 h, including sampling (using gauze), sterilization, clarification (3500 rpm x 5 min) and DNA extraction (Maxi Kit E.Z.N.A.). The NGS reached a specificity of 100% toward M. bovis DNA in detection assays carried out with artificially contaminated saliva, comparing against DNA from control microorganisms including environmental mycobacteria (M. avium, M. kansasii, M. gordoneae, M. scrofolaceum), Escherichia coli and a microbial consortium grown from cows' saliva. The detection limit in these experiments was of 0.1 femtomoles (125 ng DNA from M. bovis), proving a sensitivity close to PCR assays.

The NGS showed a strong analytical performance and has thus the potential to be validated through field assays by comparing its diagnostic accuracy against tuberculin test and qPCR. In addition, the prototype combines portability, rapid response and other advantages associated with a digital devise, such as automation, objectivity derived from the quantitative detection principle and the employment of a software allowing straightforward result reading (mobile app). These results support the development of a novel technology platform for on-site diagnostic of bovine tuberculosis and for other diseases in the future.

Acknowledgments

We thank Fundación Copec-UC (Grant 2020.R.79) and the Servicio Agrícola y Ganadero (SAG) for valuable contributions and orientations.

References

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