Main topic : Feedback on the role of veterinary laboratories in the COVID-19 crisis

Design and Validation of an In-House Real time RT-PCR for SARS-CoV-2 detection for domestic animals with targeting in nucleocapsid protein gene

ZANABRIA K. [1], OVIEDO N. [1], NAVARRO D. [1], RODRÍGUEZ S. [1], MANCHEGO A. [1], LAZARO DE LA TORRE C. [4], SANTOS N. [2], LLANCO L. [3], ROJAS M. [1]

[1] Laboratorio de Inmunología y Virología Veterinaria, Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima, Peru; [2] Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; [3] Escuela de Medicina Humana, Universidad Privada San Juan Bautista, Chincha, Peru; [4] Laboratorio de Farmacología y Toxicología Veterinaria, Facultad de Medicina Veterinaria, Universidad Nacional Mayor de San Marcos, Lima, Peru

Introduction: SARS-CoV-2 has spread worldwide and become a public and animal health problem. Consequently, the demand and cost of inputs for diagnostic tests increase. Developing countries like Peru have difficulties expanding their ability to purchase PCR tests. Therefore, developing sensitive and specific in-house methods is essential to improve the epidemiological surveillance of these countries. This study aimed to develop and validate a qRT-PCR targeting the N gene of SARS-CoV-2 using a synthetic positive control (200,000 copies/µL of the N gene) and negative controls of the genera ?-CoV, ?-CoV, ?-CoV, ?-CoV. **Methods:** We used bioinformatics tools to design the primers and the specific fluorescent probe (FAM); the amplified product was 138 bp. Endpoint RT-PCR was first performed to confirm that the primers work. Next step, the performance of the qRT-PCR was validated using serial dilutions in base 10 of the positive control in triplicate to calculate the standard curve, thus determining the sensitivity, specificity, and efficiency of the test. At the same time, a commercial kid 2019-nCoV RUO from the CDC was performed to compare our results. Finally, our test was validated using SARS-CoV-2-positive cat and dog fecal samples confirmed by sequencing. **Results:** The proposed in-house qRT-PCR presents an efficiency of 92.1%, with a minimum detection of 100 gene copies per sample, values similar to the CDC commercial kit that shows an efficiency of 98% and 89% with a minimum detection of 100 copies per sample. Commercial and in-house qRT-PCR detect SARSCoV-2 with similar results when evaluating samples from positive dogs and cats. Under current Peruvian conditions, this method generates approximately 60% savings compared to the costs of commercial qRT-PCR; also, the Peruvian market does not have a molecular test for SARS-CoV-2 detection in animals. **Conclusion:** The in-house qRT-PCR shows satisfactory results for SARS-CoV-2 detection and can be applied as an alternative test method for epidemiological studies and correlations between species.