

**Bovine coronavirus as a model for the evaluation of products with antiviral activity****Carmen Laura Perera González<sup>1</sup>, Ana María Acevedo Beiras<sup>1</sup>,  
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**Citation:** Bovine coronavirus as a model for the evaluation of products with antiviral activity. Am J of Viro and Dis. 2020; 2(3): 01-02.

**Submitted:** 23 July 2020; **Approved:** 31 July 2020; **Published:** 02 August 2020

**To the Editor,**

Humanity has been affected by a variety of infectious diseases and some of them have become in pandemics. In the 21st century, human epidemics caused by viruses, including those caused by coronaviruses, have continually appeared in the world. In less than 20 years, three zoonotic coronaviruses have emerged: the Severe Acute Respiratory Syndrome (SARS), caused by SARS-CoV in 2002 (1), the Middle East Respiratory Syndrome, caused by MERS-CoV in 2012 (2) and the COVID-19, caused by SARS-CoV-2 in 2019 (3).

The rapid transmission of SARS-CoV-2 has led many research groups to work on the detection and development of antiviral products. To determine the antiviral activity of a compound, studies that closely simulate the real conditions of virus infection are required and in vitro models with cell cultures are widely used for purpose. These experimental systems are very useful in research, since they allow the application of analytical techniques that cannot be used in humans for ethical reasons (4).

Before submitting a new product application to the regulatory authorities, it is necessary to go through the pre-clinical research stage. Pre-clinical studies are a crucial stage in the development of these new products, since they provide the scientific evidence required by the regulatory bodies so that the new product can enter a clinical phase.

To test the inhibitory activity and toxicity of a new antiviral agent in the preclinical phase, the recommended systems to be used are cell cultures (5). Viruses vary considerably in their ability to replicate in cultured cells, so the first thing to do is to select the cell system where the replication of the virus can be measured. The activity of the new antiviral agent can be assessed by the ability of the virus to cause a cytopathic effect in the culture cells, by the formation of plaques or by the ability of some viruses to produce specialized functions such as haemagglutination, hemadsorption or transformation of the cells.

In this communication it is proposed an in vitro model based on cell cultures, which allows the evaluation of the antiviral activity of both natural and synthetic products under development. The MDBK cell line and a strain of bovine coronavirus capable of producing a cytopathogenic effect (CPE) on these cells are used. The model virus belongs to the family *Coronaviridae*, to the subfamily *Orthocoronavirinae*, to the genus *Betacoronavirus*, as well as to the pandemic virus SARS-CoV-2.

The method consists of evaluating the antiviral activity of the compound based on its ability to prevent CPE caused by the virus in the MDBK cell line. Different concentrations of natural or synthetic products are evaluated and the lowest concentration of the product that inhibits virus replication is determined.

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CPE is visualized by microscopic observation of cell culture monolayers. If it is evident that the product is causing a reduction in virus yield, virus titration is performed by diluting the end point in 96-well microplates. Eight dilutions of the test compound are analyzed and the effective antiviral concentration is determined by regression analysis.

This virus has the additional advantage that it can be cultivated in the laboratory at a Biosafety Level 2 (BSL2) and does not cause any negative effect in people; unlike SARSCoV-2, which requires biosecurity measures for viral culture, under BSL3 conditions.

Taking into account the advantages of this model mentioned above, we consider its use to evaluate natural and synthetic products with potential antiviral activity, which could be included in the future as part of the arsenal of drugs science is developing for the prevention or treatment of COVID-19.

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