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Short communication

An improved method for determining virucidal efficacy of a chemical disinfectant using an electrical impedance assay

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A B S T R A C T

A major problem with the testing of virucidal efficacy using current protocols is that scoring of virus-induced cytopathic effect (CPE) is dependent on subjective visual interpretation using light microscopy. The current report details the use of an electrical impedance assay (xCELLigence, ACEA Biosciences) for its utility in virucidal efficacy testing. In this study, the xCELLigence system was used in a procedure developed from guidelines given by the Deutsche Vereiniging zur Bekämpfung der Viruskrankheiten (DVV) (German Association for the Control of Virus Diseases) in order to demonstrate the inactivation of infectious bursal disease virus using a commercial virucide. Although the modified DVV assay using the xCELLigence system yielded identical results (i.e. a 5-log 10 reduction in viral infectivity) as the traditional DVV assay, the system allows virucidal efficacy and cytotoxicity to be measured in a more precise and reproducible fashion.

Regular cleaning and disinfection to reduce the level of bacterial and viral pathogens is essential in the domestic farming environment and veterinary and medical practices (Kassaify et al., 2007). There are currently two methods that are widely used for testing the efficacy of virucidal compounds, i.e. the viral carrier and suspension tests. The viral carrier test attempts to determine viral inactivation on hard surfaces (instrumentation, hands, or other surfaces) following contact exposure to a virucidal compound of interest, whereas in the suspension test, virus is incubated together with the virucidal compound in a liquid suspension. Both methods are dependent on evaluating viral inactivation by observing the development of viral cytopathic effect (CPE) in permissible cell cultures (Bellamy, 1995).

In Europe two methods are available for viral suspension testing, the German DVV (Deutsche Vereiniging zur Bekämpfung der Viruskrankheiten) (Blümel et al., 2009) and French AFNOR (Agence Francaise de Normalisation) (AFNOR, 1989) tests. Both methods assume virucidal efficacy if a 4-log 10 reduction in viral titre occurs following compound exposure, although this reduction in viral infectivity cannot always be demonstrated due to the cytotoxicity of many compounds in cell cultures at lower dilutions (Bellamy, 1995).

A major problem with virucidal efficacy testing is that there is currently no broadly accepted universal standard, which makes it difficult to compare results between laboratories. In particular, the scoring of CPE is based on the visual interpretation of cell culture deterioration using light microscopy, which is subjective. Recently a new method has been developed for measuring virus-induced CPE by using an electrical impedance system (xCELLigence, System, ACEA Biosciences) (Spiegel, 2009). This system uses specially coated cell culture plates, called E-plates, in which the wells are coated at the bottom with gold micro-electrode sensor arrays. The system can be used to visualize virus-induced CPE in a software assisted manner, in real-time, by measuring changes in electrical impedance (CI, cell index value) in infected cell monolayers.

The current report describes the use of the xCELLigence system in a modified DVV test, in order to evaluate the inactivation of infectious bursal disease virus (IBDV, genus *Avibirnavirus*, family *Birnaviridae*) using a commercial virucide.

The virucide testing was carried out in Vero monolayer cultures (Vero 76, American Type Culture Collection, Manassa, USA). Vero cells were propagated in Minimal Essential Medium (MEM) supplemented with 5% gamma-irradiated fetal calf serum (Highveld Biological, Edenvale, South Africa), 10% tryptose phosphate broth (v/v) and 1 mg/L gentamicin (Highveld Biological). Cells were maintained at 37 °C and 98% humidity until 80% confluent. In order to prepare a virus stock, a field isolate of IBDV was sequentially passaged four times in 80% confluent Vero cells using 75 cm^2 flasks (Corning). Once 100% CPE was observed at the final passage, the cell culture flask was frozen at −80 °C, thawed and the virus-containing cell culture supernatant separated from cell debris by centrifugation at 2000 × *g* for 2 min. The virus containing cell culture supernatant was subsequently titrated in Vero cell cultures and viral titres expressed as $\log_{10}$ $TCID_{50}$/mL of cell culture medium

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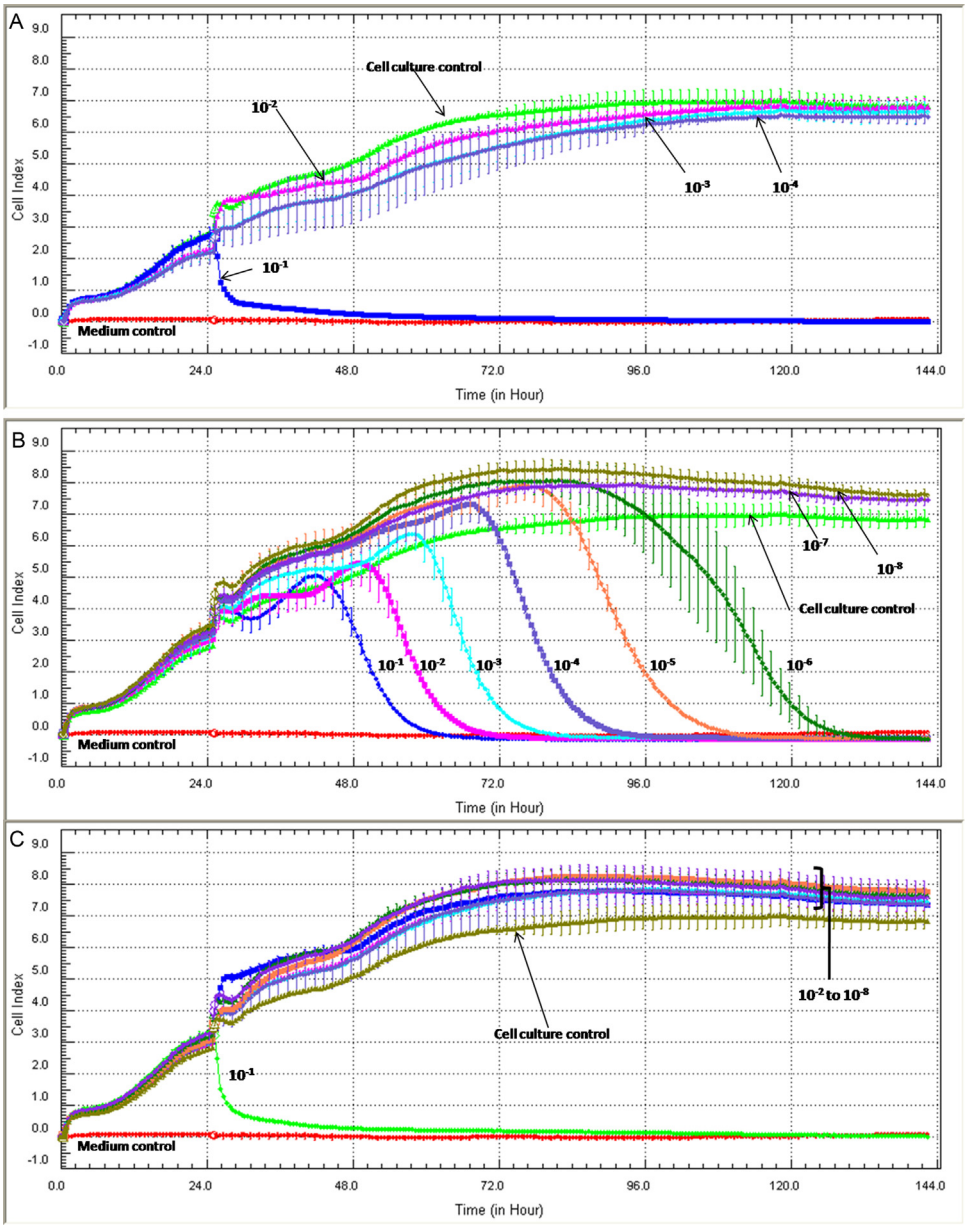

Fig. 1. (A) Virucide mixture. (B) Virus control mixture. (C) Virucidal test mixture. Cytopathic effect of (A) different virucide control dilutions, (B) virus dilutions, and (C) virus–virucide mixture dilutions on Vero cells (DVV assay) as scored using the xCELLigence system. Each curve is composed of CI values from three independent replicates. Cell index values were measured at 30 min intervals following mixture addition. A CI value of zero indicates complete detachment of cells from the bottom of the cell culture wells. Cytotoxicity of the virucide control mixture (A) was measured only from 10^{-1} to 10^{-4} dilutions. Standard deviations are indicated for each reading/curve by error bars.

(Kärber, 1931). The virucidal compound was obtained from a commercial supplier and tested at the recommended active dilution and contact period (20 min) according to the manufacturers' specifications.

For comparative purposes, the DVV assay was conducted using both a conventional method, in which CPE was scored visually, as well as by using the xCELLigence system. In order to prepare the assays, 96-well E-plates were seeded with 80 μl of a 480 000 cells/mL suspension (38 400 cells) and allowed to grow to confluence overnight. Cytotoxicity control, virus control and virucidal test mixtures were prepared. The first concentration of the virucidal compound used was at 1.25 times the recommended test concentration. The virus control mixture consisted of 6-$\log_{10}$ $TCID_{50}$/mL virus in MEM. The virucidal test mixture consisted of a 1:5 dilution of virus in the virucidal compound. All mixtures were incubated at room temperature for 20 min (contact time), before being serially diluted (eight tenfold dilutions). The 96-well E-plates were divided into three equal parts for the three mixtures and 200 μl of the appropriate dilutions of the mixtures added at approximately 24 h post cell seeding. Three wells were used for each dilution. Cell culture and cell free medium controls were included.

The plates were incubated for seven days at 37 °C in an atmosphere containing 5% CO_2 and CPE scored either by visual interpretation using light microscopy at 7 days post mixture addition (conventional DVV assay) or cell index (CI) values measured every 30 min on the xCELLigence system (RTCA-SP-96). For the xCELLigence assay, a reduction in cell index value of 50% (CI_{50}) was used as an estimate of the rate of induction of cell death. Virucidal activity was calculated by comparing the titre of the virus in