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Establishment of A Highly Sensitive Assay System for Screening of Anti-Orthomyxo- and -Paramyxoviruses Agents. Wataru Watanabe*, Kenji Konno*, Tomoyuki Yokota* and Shiro Shigeta**. *Rational Drug Design Laboratories, Fukushima (960-12) and **Department of Microbiology, Fukushima Medical College, Fukushima (960-12), Japan.

We established an easy and highly sensitive screening system for antiviral agents against orthomyxo- and paramyxoviruses. One of the technical key points of our assay system is to concentrate the cells on the bottom of 96-well round bottomed microtiterplates by a low speed centrifugation to lead efficient virus infection in the cells. The assay system is based on detecting cellular dehydrogenase activity by MTT method. The cell lines used for the assay were as follows; MDCK cells for influenza A virus (Fluv. A), HeLa cells for RS virus (RSV) and Vero cells for measles virus (MSV). Diluted test compounds were plated in the microplates. The trypsinized cell suspensions and viruses were then added to each well in the plates. After centrifugation (700xg, 5 min, RT), the plates were incubated for several days at 35 C in a humidified 5 % CO_2 incubator. MTT assay was performed following recognition of the virus-induced cell degeneration by microscopy. The optical density (OD) of formazan was read in a computer-controlled microplate reader and the results were calculated automatically. The EC_{50} values of ribavirin, which was used as the reference compound, were 2.0 μg/ml for Fluv. A, 4.2 μg/ml for RSV and 10.8 μg/ml for MSV, respectively. These EC_{50} values were equivalent to the results obtained by plaque reduction assay. The EC_{50} values of several anti-RNA virus agents (i.e. pyrazofurin, carbodine, dextransulfate and other) were also similar to those of plaque reduction assay. The confluented cell culture system was inadequate for antiviral assay against RSV or MSV using MTT method, because the significant difference on OD value was not observed between virus-infected cells and mock-infected cells. Thus, the assay system established in our laboratories can be available for an automatic screening system for antiviral agents against various myxoviruses.

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Fluorometric ELISA Method For Rapid Antiviral Susceptibility Testing of *Respiratory Synctial Virus*. **S.F. Reising, K. Quinn, S. Lindeman and J. Doughman. Children's Hospital Research Foundation, University of Cincinnati, Cincinnati, Ohio, U.S.A.**

We have developed a micro-fluorometric based ELISA for determination of *in vitro* susceptibility of existing or newly developed antiviral agents. For RSV isolates, confluent monolayers of HeLa cells in a 96-well microtiter plates format were infected with various concentrations of virus. Infected monolayers with and without ribavirin were incubated for 30 to 48 hours. Cells were lysed with 1.2% sodium dodecyl sulfate and the contents of the wells (200 ul) transferred to a 96-well surface modified nylon membrane with positive zeta potential. This maximizes the ability to capture both free and cell associated virus. This is of importance for viruses that are not highly cell associated. Viral antigen was measured utilizing pooled mouse monoclonal antibodies directed against the RSV 47 Kd fusion protein, the 89 Kd glycoprotein and the 43 Kd nuclear protein. Phosphatase labelled sheep anti-mouse IgG was used as the secondary antibody and 4-methylumbelliferyl (MP) as the substrate. Fluorescence was measured by Microflour fluorometer at excitation 360 nm wavelength and emission 450 nm wavelength. This system offers a simple, sensitive, reproducible and rapid method for RSV antiviral susceptibility testing. In addition, this format offers the ability to test other viral agents and antiviral compounds.