Direct immunofluorescence (DFA) on HEP2 and R-Mix Too for Adenovirus detection

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Immunofluorescenza diretta (DFA) su cellule HEP2 e R-Mix Too per la rilevazione degli Adenovirus

INTRODUCTION

Human adenoviruses (ADV) are nonenveloped, icosahedral viruses containing a single linear, double-stranded DNA genome.

ADV can cause different clinical syndromes in immunocompetent individuals. High incidence of adenoviral infections and a severe clinical impact were observed in the immunocompromised patients, in particular ADV has been associated with airway diseases (1).

One of the classical diagnostic tools for ADV detection in biological samples is represented by immunofluorescence.

Cell lines commonly used include human epithelial cells such as A549, HEP2, the latter most often (2). R-Mix Too are a mixed cell cultures, consisting of A549 and MDCK cells, useful to detect the presence of respiratory viruses in biological samples in 24-48 hours. The purpose of this study was to compare the detection of ADV by direct immunofluorescence on HEP2 and R-Mix Too cells.

MATERIALS AND METHODS

Ten-fold dilutions of ADV from 10^4 $TCID_{50}$/200µl to 10^{-2} $TCID_{50}$/200µl were used to infect both HEP2 and R-Mix Too shell vials (Diagnostic Hybrids).

Immunofluorescence was performed using 3 different antibody dilutions (1:40, 1:80 and 1:160) and results were analyzed 48, 72, 96 hours post infection for HEP2 cells and 24, 48 hours for R-Mix Too cells according to the manufacturer's instructions.

RESULTS

ADV positivity was observed up to 10^{-1} $TCID_{50}$/200µl on HEP2 cells (72h post infection with 1:40 antibody dilution), with a sentitivity of 10^0 TCID50/200µl. As regards R-Mix Too cells, ADV positivity was observed up to 10^1 $TCID_{50}$/200µl (48h post infection with 1:80 antibody dilution), with a sensitivity of 10^2 $TCID_{50}$/200µl.

ADV detection	Cell Type	
	HEP2	R-Mix
Too LOD (Limit Of Detection: $TCID_{50}$/200µl)	10^{-1}	10^1
Sensitivity ($TCID_{50}$/200µl)	10^0	10^2
Time post infection (hours)	72	48

CONCLUSION

The comparison among two different cellular substrates for the detection of ADV has showed a difference of two logarithms in terms of sensitivity. In fact HEP2 cells are resulted more suitable as the sentivity reaches 10^2 $TCID_{50}$/200µl instead of 10^0 $TCID_{50}$/200µl for R-Mix Too cells.

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