A Novel Rapid Fluorescent Focus Inhibition Test for Rabies Virus Using Recombinant Rabies Virus Visualizing a Green Fluorescent Protein

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Virus-neutralizing antibodies (VNAs) against rabies virus play a major role in protection from rabies. The rapid fluorescent focus inhibition test (RFFIT) has been internationally recognized as a standard in vitro test for measuring the VNA. CVS strain of rabies virus is used as challenge virus and the infected cells are indirectly detected by staining with fluorescein isothiocyanate (FITC) -conjugated rabies antibody which is expensive and high-quality products are often in short supply. In this study, a recombinant rabies virus strain carrying a green fluorescent protein (GFP) gene, rHEP-GFP, was used as a challenge virus in the virus neutralization assay. Expression of the GFP could be readily detected in the infected cells under a fluorescent microscope. This novel RFFIT modification RFFIT-GFP is a neutralization test, and it is based on the sound principle of the standard RFFIT using 96-well plates. VNA titers in 25 human, 18 canine and 15 horse sera have been compared between the RFFIT and RFFIT-GFP methods. The results obtained by the both methods showed good agreement between both methods in all sera investigated (coefficient of correlation = 0.98). It allowed direct detection of virus by expression of GFP and might be applicable for other viruses. The novel method is convenient, economical and reliable tool not requiring expensive FITC-conjugated antibody for routine rabies VNA assays.