Enzyme-Linked Immunosorbent Assay-Format Tissue Culture Infectious Dose-50 Test for Titrating Dengue Virus

Jie Li1,2, Dong-mei Hu1,2, Xi-xia Ding1,2, Yue Chen1,2, Yu-xian Pan1,2, Li-wen Qiu1,2, Xiao-yan Che1,2*

1 Center for Clinical Laboratory, Zhujiang Hospital, Southern Medical University, Guangzhou, People’s Republic of China, 2 Key Laboratory of Prevention and Control of Emerging Infectious Diseases of Guangdong Higher Education Institutes, Guangzhou, People’s Republic of China

Abstract

A dengue nonstructural protein 1 (NS1) antigen capture enzyme-linked immunosorbent assay (ELISA)-based tissue culture infectious dose-50 (TCID\textsubscript{50}) test (TCID\textsubscript{50}-ELISA) was developed as an alternative to the standard plaque assay for titrating dengue virus. Virus titers obtained by TCID\textsubscript{50}-ELISA were comparable to those obtained by the plaque assay and by the traditional TCID\textsubscript{50}-cytopathic effect (CPE) test (TCID\textsubscript{50}-CPE), with a better reproducibility and a lower coefficient of variation. Quantitative comparison of TCID\textsubscript{50}-ELISA and TCID\textsubscript{50}-CPE resulted in a correlation coefficient of 0.976. Moreover, this new method showed a wider applicability to C6/36, Vero E6, BHK-21, and Vero cells compared with other titration methods. In summary, the novel TCID\textsubscript{50}-ELISA method described here provides a more reliable and more accurate alternative compared to the plaque assay and TCID\textsubscript{50}-CPE for titration of dengue virus.

Introduction

Over the past 60 years, dengue fever and dengue haemorrhagic fever have become increasingly serious public health problems in the tropics and subtropics due to overpopulation, ever-increasing regional and international travel, as well as global warming [1]. Dengue virus (DENV), a member of the family Flaviviridae, genus Flavivirus, is an enveloped, single-stranded RNA virus comprising four antigenically distinct serotypes: DENV1, DENV2, DENV3, and DENV4. Infection with any one of the four serotypes can result in a broad spectrum of consequences, including asymptomatic infection, mild febrile illness, classic dengue fever, and the lethal dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) [2]. Research on DENV is often hindered by inefficient and inaccurate or costly viral titration methods [3]. Hence, a simple and efficient assay for accurate titration of DENV in infected cultures would greatly facilitate dengue research, vaccine development, and laboratory detection. To date, a variety of methods for titrating DENV have been developed, including classical assays, the plaque assay and the tissue culture infectious dose-50 (TCID\textsubscript{50}), and immunofluorescence-based assays such as fluorescence-activated cell sorting (FACS) assay and fluorescent focus assay [4,5,6,7]. As a standard method for titrating DENV, however, the plaque or TCID\textsubscript{50} assays have their disadvantages, as they are limited to some strains and passages of the virus, and a few cell lines [5]. Most primary clinical isolates do not form clear plaques or have a visible cytopathic effect (CPE) on cell monolayers. Furthermore, both of these assays require manual microscope examination daily, which is time consuming and labour intensive. FACS and fluorescent focus assays can provide more rapid and accurate quantitation of DENV than the traditional plaque assay [4,6]. However, each of these techniques requires experienced technicians and sophisticated laboratories, hindering its application in most laboratories lacking sophisticated equipment. Therefore, convenient methods for titrating the virus need to be developed. Nonstructural protein 1 (NS1), a multifunctional glycoprotein in dengue virus, is highly conserved for all serotypes of DENV and is strongly immunogenic [8]. Some of the NS1 protein is expressed as a soluble secreted form, which has been implicated to contribute to dengue viral propagation and the amount secreted is closely related to dengue viral titer [9]. In our previous study, we established a dengue NS1 antigen capture enzyme-linked immunosorbent assay (ELISA) [10]. In the present study, a novel TCID\textsubscript{50} assay was developed, which employs this dengue NS1 antigen capture ELISA for the accurate and objective titration of DENV.

Materials and Methods

Cells and viruses

*Aedes albopictus* cells (C6/36, ATCC:CRL-1660) were maintained in minimum essential medium (MEM; Gibco) supplemented with 10% fetal bovine serum (FBS; HyClone Laboratories, Logan, Utah) and 0.1% gentamicin (50 μg/mL, Sigma Chemical Co., St. Louis, Mo.) at 28°C or 37°C in 5% CO\textsubscript{2}. African green monkey kidney cells (Vero E6, ATCC:CRL-1586), Hamster kidney cells (BHK-21, ATCC:CCL-10) and African green monkey kidney cells (Vero, ATCC:CCL-81) were maintained in MEM...