CHAPTER I

INTRODUCTION

Laboratory diagnosis of viral infection can be made of either by demonstration of the presence of virus, viral antigen or virus-specific nucleic acid sequences, or by measurement of viral antibodies (1,2). The diagnosis of viral diseases has difficulty in demonstration of the organism is expensive and laborious, as the host of the viral propagation are very specific, for example, the tissue culture, experimental animal or embryonated egg. The serological methods are playing an increasingly important role in the diagnosis and epidemiological assessment of diseases. However, currently used assays, primarily complement fixation test (CF) and neutralization test (NT) are not satisfactory in all respects (3,4). At present, there are two widely accepted assays that employ labelled antibodies and antigens. They are immunofluorescent antibody assay (IFA) in which a fluorescent dye is conjugated to the antibody, and radioimmunoassay (RIA) in which isotopes are attached to antibodies or antigens. Practically, immunofluorescence is not easy to quantify for antibody assays. Although RIA is very sensitive and permits precise-quantification, its widespread use is limited by the inherent problems of the hazard of the isotopes employed. Therefore, simple and

inexpensive method for a large-scale application is urgently needed. The enzyme-linked immunosorbent assay (ELISA) developed recently and reviewed elsewhere (5,6,7,8) hold great promise in a wide variety of applications. It has been accepted that ELISA is as sensitive as radioimmunoassay (3,9), but more simple, and can be adapted to be used as the field screening procedure.

In this study, herpes simplex virus (HSV) was used as a model for preparing ELISA antigen because it can be easy to propagate in tissue-culture. In addition, HSV has a worldwide distribution and is common pathogen which infects man and wide-range animals. Although, the infections are frequently mild with localized lesions. In rare occasion, the infection is severe and results in serious damage that may cause death of the host, especially in the intrauterine herpetic infection of the newborn (10,11,12).

Herpes simplex virus is a member of the alpha subgroup of the herpesvirus family. The herpesviruses that commonly infect man are herpes simplex virus type 1 and 2 (HSV-1 and HSV-2), varicella-zostor virus (VZV), Epstein-Barr virus (EBV) and cytomegalovirus (CMV)(13). There is considerable homology between HSV-1 and HSV-2 genome; about 50 percent of nucleic acids, whereas there is little homology between any other two herpesviruses (14).

Generally, HSV-1 is transmitted in oral secretions, while HSV-2 is transmitted genitally. It is agreed that most oral isolates of HSV are of the type 1 (oral-type), while genital isolates usually represent HSV type 2 (genital-type) (15,16).

Human is the natural reservoir of HSV. Since the virus cannot remains infectious for a long time outside the body, direct contact with infected secretion is the mode of the spread of the virus. Most primary infections go unrecognized or are subclinical (17). HSV becomes latent following a primary infection by travelling up the sensory nerves to the corresponding sensory ganglia. The usual clinical manifestation is a vesicular eruption of the skin or mucous membranes. Infections are sometimes severe such as keratitis, meningoencephalitis, and a disseminated illness of the newborn (18,19). There are some evidences showing the closed relation between HSV-2 and cancer of the cervix (20,21). The virus has thus been the subject of much attention, particularly over the past decade.

In ELISA , the antigen used for the detection of antibody must be specific. In developing countries, if the antigens have to be purchased, the assay which used in the detection of antibody will cost enormously. Therefore, it is necessary to work-up on the methodology of antigen preparation which is efficient in terms of

simplify, accuracy, specificity, and low cost of the production.

The purpose of this study was to determine the suitable method for producing HSV-ELISA antigen. The antigen was then used for the detection of antibody to HSV by the ELISA procedure and then, the ELISA method developed in this study was compared to a commercial ELISA kit and also to immunofluorescent antibody technic. In addition, the preservation and the stability of the ELISA antigen in different preparations were also to be evaluated.