CHAPTER V

DISCUSSION

A number of studies have shown that ELISA is sensitive, specific, and favorably compared to the other more established immunological technics (3, 4, 96). In our studies, we attempt to find out the suitable method for preparing HSV-ELISA-antigen because HSV can be propagated in several cell lines (3, 27,47, 81, 82, 83) and produce infectious which are expressed in a variety of clinical manifestations ranging from mild to fatal diseases (11, 32, 54, 56, 59, 62). Moreover, the published literatures indicated that the ELISA technic was a useful addition to the wide range of currently available methods for detection of antibody to HSV (3, 4, 82, 83, 84, 85). The results of the present investigation demonstrated that the ELISA antigen, crude extract of infected cell, was high efficiency for detecting antibody. The use of crude antigen eliminated laborious and time consuming procedures required for purification of virus. These considerations, together with the fact that only cheap and simple equipment need to be used. Accordingly, this method for preparing of antigen is simple, economical, and especially suitable for use in small laboratories and in developing country.

Preliminary experiment, crude antigen was prepared from HSV-infected Vero cells by two methods. As shown in Table 3, the antigen in PBS is better than the antigen in MEM without serum, in addition the antigen in MEM without serum caused higher nonspecific reaction. This is because protein in MEM consist of antigen and medium so, the quantity of antigen in MEM is less than in PBS when the protein concentrations are equal (40 µg/ml). Later, the preparation of antigen in two cell lines, HeLa and Vero cells were comparatively studied. The results showed that antigen which prepared from HeLa cells is more suitable than that of from Vero cells. Although, other investigators (83, 84, 85) were successfully employed Vero cells in preparing of antigen. It is possible that our conditions for testing or preparing of HSV-antigen is more suitable in HeLa cells. These prepared antigens were inactivated by destroying the infectious particle before use. Various methods were tried out such as ultraviolet, formalin and heat inactivation (91). Heat was found to be effective and also was a convenient method in killing the virus. Although, the virus was completely destroyed in a waterbath at 50 ^{o}C for 15 min, but the antigen gave the highest efficiency at 50 ^{o}C for 1 h. The longer period of heating may alter the protein properties which in turn give the low background of cell control.

The ELISA used many kinds of reagents of which the best result depended on the proportion of each reagent-

concentration. The optimal concentration of antigen, antibody, and conjugate can be determined by checkerboard titration (85, 94, 95). Such a titration was illustrated in Table 5. The suitable conditions are that the optimal concentration of antigen was 80 µg/ml, and the dilution of antibody and conjugate were 1:50 and 1:2,400, respectively. In our study, we decided to use the patient serum at dilution of 1:100 because the dilution of 1:50 gave nonspecific reaction higher than that of the former.

Whenever any immunologic assay was developed it must be standardized very strictly. Without such standardization, the developed assay cannot count to be valid. Standardization of the assay was indicated by precision and specificity analysis of that test. The reproducibility of the test determine by coefficient of variation. In our ELISA test it was found that the CV was ranging from 9.56 to 13.58 %. Several workers (73, 74, 80, 97) found immunological relationship between HSV and VZV. But, there was no cross reaction between our HSV-antigen and antibodies to other viruses in herpesvirideae.

The developed ELISA showed high specificity and sensitivity when compared with commercial ELISA kit (Table 10). The specificity was 100 %. It may be due to the dilution of serum which was 1:100 while the commercial kit dilution of serum was 1:44. The lower dilution may be prone to cause non-specific reaction. The sensitivity of

developed ELISA was as high as 92.5 %.

In Table 11, the developed ELISA was compared with IFA which is a routine assay at Chulalongkorn hospital. It was found that the developed ELISA was comparable to the IFA method in with sensitivity and specificity. However, IFA method is laborious and time consuming. So, the developed ELISA may replace the IFA in the future.

The stability was tested in various conditions from 1 to 6 months period (Table 12). It was found that the suspension kept at -20 ^{o}C gave the same result as the original while the lyophilized showed nearly the same finding. The antigen coated on solid phase kept for 6 months showed that only 2 cases gave different results (Table 12.3 and 12.4). No. 33 of the antigen coated on solid phase and kept at -20 ^{o}C, the result switched from negative to positive (the original O.D. is 0.180 and after kept at -20 ^{o}C, the O.D. are 0.378, 0.357, 0.267 and 0.365 for 1, 2, 3 and 6 months, respectively). The result of serum No.32 when react with the antigen coated and washed before kept at -20 ^{o}C, switched from positive to negative (the original O.D. is 0.222, and after washed before kept at -20 ^{o}C, the O.D. are 0.159,0.198, 0.190, 0.182 for 1, 2, 3 and 6 months, respectively). It may be due to both of the sera were in borderline.

Our work agreed with those of other workers, who found that crude antigen can be used for ELISA test (73,

76, 81, 84, 86).

The commercial ELISA kit is composed of the two coated plates and kit-solutions. It cost 10,500 bahts per 92 tests. The price of a single coated plate is 2,500 bahts which is rather expensive. The same kind of this plate prepared in our laboratory with antigen (local-made) costs much cheaper.

The ELISA is a useful test due to its rapid and considered as one of the standard methods (5, 6, 7, 8). This research is the model in the way to prepare other ELISA antigens. It may also be adapted to prepare other ELISA viruses antigen such as CMV and EBV once the technology has been established.

Besides, the ELISA can be used in research, extensively. Buying antigen coated plate and other expensive accessories would be a financial problem. Therefore, the availability of local-made antigen will help to solve this problem. To study the end point of antibody in serum sample, i.e. the antibody titer of normal persons and patients, or the differences in titers of antibody in acute- and convalescent serum. This such elaborate work would be impossible if the coated plated has to be purchased.

It is suggested that the research on determining anti-HSV (IgM) that can support the diagnosis of acute

infection should be carried out. Initial study for, detection of anti-HSV IgM has been tried. The result was not conclusive. It could be explained in three ways : 1) lacking of strong positive serum for anti-HSV-IgM, 2) interference of the high levels of IgG antibodies in the patient's sera, and 3) the antigen is not suitable for detecting IgM antibodies. The determination of specific IgM antibodies is needed for diagnosis of viral diseases. The further study should be the improvement of antigen preparation and reestablishment of the ELISA system.