Thesis Title : Detection for Hepatitis Be Antigen and Antibody to Hepatitis Be Antigen by Microelisa System

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Abstract

Hepatitis B e antigen (HBeAg) and its corresponding antibody (anti-HBe) are useful for predicting infectivity of serum and the follow up of chronic hepatitis B. Moreover, they are most useful in screenining pregnant women in which the presence of HBeAg indicate active , and if possible , in combination with passive immunization in the newborn infants. Presently both tests have to be imported at high cost which limit their application . The key reagent for the test is human high titer anti-HBe which is locally available. The objective of the project was the local production of the two tests using local raw material. The sensitive, spicific and reproducible microelisa for HBeAg and anti-HBe were successfully developed using human anti-HBe of extremely high titers, obtained from Thai blood donors. The IgG fraction of human anti-HBe was diluted in 0.02 M tris buffer pH 7.6 and used to coat polystyrene microtiter strip in the capture layer. IgG anti-HBe coupled to HRP by the periodate technic, was added, incubated

for 1 hour and washed. OPD was added and incubated for 30 minutes and the reaction stopped with $4NH_2SO_4$. The methods were sandwich ELISA test for HBeAg and the blocking ELISA for anti-HBe. The test were compare with two commercial ELISA tests for HBeAg and anti-HBe. Comparison between the developed and Organon's ELISA in 103 HBsAg carrier, positive for either HBeAg or anti-HBe was found in 96% and 94% by the developed and Organon's tests respectively and there was an agreement of 97.1%. Comparison between the two tests in 205 routine specimens for HBeAg and anti-HBe showed an agreement of 97.5 %. A similar comparison between the developed and Roche's ELISA tests for both markers in 160 routine specimens showed an agreement of 98.1%. Interference by rheumatoid factor was reduced to minimal and the present test showed good reproducibility and stability. These tests can replace imported kit without sacrificing quality, and make it possible for most hospital to use this low cost test for diagnosis, monitoring and control of hepatitis B infection in Thailand.