

DEVELOPMENT OF AN ELISA KIT FOR DETECTION OF HEPATITIS B SURFACE
ANTIGEN IN PLASMA AND SERUM, BASED ON POLYCLONAL ANTIBODIES
GENERATED IN
KEMRI, KENYA

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ABSTRACT

Hepatitis B infection is a disease of the liver caused by Hepatitis B Virus (HBV), a double-stranded DNA virus coated with an envelope containing Hepatitis B surface antigens (HBsAg). HBsAg levels in blood are high as long as the viral particles continue to exist in the liver cells. Hence they are the most important markers used in screening for the presence of Hepatitis B infection in many of the diagnostic test kits in the market. The currently available ELISA diagnostic kits for HBV are both imported and expensive.

The main objective of this study was to develop a cost-effective Enzyme-linked Immunosorbent Assay (ELISA) kit for detection of HBsAg in plasma and serum using polyclonal antibodies produced in KEMRI.

The capture polyclonal antibodies were obtained from KEMRI Production department where they had been raised in guinea pigs inoculated with locally prepared HBsAg. The Sandwich-based ELISA system was prepared by coating capture antibodies on 96-well microplates and blocking the void spaces using the Bovine Serum Albumin and Tween 20 (BSAT) blocking buffer after which samples were applied. The Horse Radish Peroxide (HRP)-linked ovine HBsAg detection antibodies were incubated in the wells and washed with Tween 20 Wash Buffer to remove the unbound antibodies. The 3, 3', 5, 5' tetramethylbenzidine (TMB)-hydrogen peroxide substrate was incubated for a 30 minutes and absorbance values read using an ELISA plate reader. The reagents used in preparation of this kit were optimized using the ELISA checkerboard technique.

The developed kit was assessed and found to have diagnostic sensitivity of 96.1%, diagnostic specificity of 100%, positive predictive value of 100% and negative predictive value of 95.7%

with Hepanostika Ultra HBsAg kit as a gold standard. The analytical sensitivity of the kit was found to be 4.62ng/ml and no analytical non-specificities were noted in samples positive for HIV and HCV respectively. The kit showed desirable repeatability profile with the coefficient of variance of intra-run repeatability of 1.38% and inter-run repeatability of 5.3% against the limit of 20%. The overall inter-observer variation agreement, Kappa statistic, was found to be 1 (one) signifying perfect correlation while the Pearson correlation coefficient (r^2) to determine correlation between values measured by different assays were 0.917, 0.939 and 1.00 against three established kits.

The ELISA kit developed in this study will be tested further in the field for a period of one year before it is applied for registration at the National Public Health Laboratory Services, Nairobi. It will also be validated against a number of mutagenic cysteine variants and subtypes on the “a” determinants of HBsAg.