UNIVERSITI PUTRA MALAYSIA

DETECTION OF HEPATITIS B CORE ANTIGEN USING A FUSION BACTERIOPHAGE

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By
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Specially dedicated to,

Papa and Mama, Kak Long, Kayna, Aji, Alol, Yan, and Limah

For their invaluable love, understanding, patience, support and care.

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SITI SALWA BINTI HASMONI

September 2005

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Due to the many reported cases of hepatitis B disease around the world, a keen interest among researches has aroused on the cause of the disease, hepatitis B virus (HBV). One of the serological markers for HBV is hepatitis B core antigen (HBcAg) that is a marker of the infectious material and it is the most accurate index of the viral replication. The importance of the HBcAg especially when considering the close relationship with the viral DNA load has created revolutionary studies on the HBcAg ever since. The HBV nucleocapsid or HBcAg is extremely immunogenic during infection and after immunization. A fusion bacteriophage that interacts with HBcAg has been isolated from a phage display peptide library. The phage interacts tightly to HBcAg and thus has the potential to be further developed as a diagnostic reagent. In this study, two immunoassays have been developed using the fusion bacteriophage to detect HBcAg. Phage-ELISA and phage-dot blot assay could detect not only purified HBcAg but also HBcAg in serum samples. As low as 10 ng of HBcAg can be significantly detected by 10^{12} pfu/ml of fusion phage when the reading at 405 nm was measured (A_{405} = 0.4). Using the fusion bacteriophage, these newly developed immunoassays

provide an easier and cheaper option for detecting HBcAg. The sensitivity of these immunoassays demonstrates the potential and perhaps vast future uses to detect HBcAg. The fusion phage is also capable of purifying the HBcAg due to its capability to precipitate HBcAg.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

PENGESANAN ANTIGEN TERAS VIRUS HEPATITIS B DENGAN MENGGUNAKAN BAKTERIOFAJ REKOMBINAN

Oleh

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Pengerusi: Profesor Madya Tan Wen Siang, PhD

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Terdapat banyak kes penyakit Hepatitis B telah dilaporkan. Ini menimbulkan minat yang tinggi kepada para penyelidik untuk menjalankan penyelidikan tentang punca penyakit tersebut iaitu virus Hepatitis B (HBV). Salah satu penunjuk serologi virus ini adalah HBcAg. Ia merupakan penanda jangkitan HBV yang sangat efektif dan paling tepat dalam menunjukkan kehadiran aktiviti replikasi virus tersebut. Kepentingan HBcAg terutama mengenai hubungan rapat protein tersebut dengan jumlah DNA HBV telah mencetus revolusi di dalam bidang penyelidikan yang telah memberi kesan yang mendalam. HBcAg atau juga dikenali sebagai nukleokapsid HBV adalah sangat imunogenik semasa jangkitan dan selepas immunisasi. Satu bakteriofaj rekombinan yang telah dipilih daripada sebuah perpustakaan pameran faj didapati dapat berinteraksi kuat dengan HBcAg. Faj yang membawa peptide-peptida tertentu ini berpotensi untuk dijadikan suatu reagen dalam bidang diagnostik. Di akhir pengajian ini, dua jenis imunoasai yang menggunakan bakteriofaj rekombinan untuk mengesan HBcAg telah berjaya dihasilkan. Faj-ELISA dan asai faj-dot blot mampu mengesan HBcAg yang telah ditulenkan dan juga HBcAg di dalam sample-sampel serum.

Sejumlah 10^{12} pfu/ml faj rekombinan berupaya mengesan dengan baik 10 ng HBcAg apabila bacaan tindakbalas yang diambil pada gelombang 405 nm diukur (A_{405} = 0.4). Imunoasai-imunoasai baru ini menyediakan suatu kaedah yang lebih murah dan mudah dalam mengesan HBcAg disamping mempamerkan pengesanan HBcAg yang sensitif. Oleh yang demikian, imunoasai-imunoasai ini berpotensi tinggi untuk digunakan di masa hadapan dalam mengesan HBcAg khususnya. Faj rekombinan ini juga boleh digunakan untuk menulenkan HBcAg, berdasarkan kebolehannya memendakkan HBcAg.

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LIST OF ABBREVIATIONS

ε	encapsidation signal
α	alpha
β	beta
µg	microgram (10^{-6} g)
µl	microlitre (10^{-6} l)
µM	micromolar (10^{-6} M)
Amp	ampicillin
anti-HBcAg	antibody to HBcAg
anti-HBeAg	antibody to HBeAg
anti-HBsAg	antibody to HBsAg
anti-HBxAg	antibody to HBxAg
°C	degree centigrade
CHAPS	3-[(3-chol-amidopropyl)-dimethylammonio]-1-propanesulfonate
CNBr	cyanogen bromide
bp	base pair
BSA	bovine serum albumin
C	cytosine/core
ccc	covalently closed circular
CTL	cytotoxic T lymphocyte
c-terminus	carboxyl terminus
DC	dendritic cell
DNA	deoxy-ribonucleic acid

DNase	Deoxyribonuclease
dNTP	deoxynucleoside triphosphate
DTT	1,4-dithiothreitol
ds	Double stranded
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunoabsorbent assay
g	Gram
h	Hour
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HBxAg	hepatitis B x antigen
HCC	hepatocellular carcinoma
HCl	hydrochloric acid
IFN	Interferon
IFN-γ	interferon gamma
IL2	interleukin 2
IL4	interleukin 4
IL5	interleukin 5
IL10	interleukin 10
IL12	interleukin 12
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IgG	immunoglobulin G

IgM	immunoglobulin M
IPTG	isopropyl-β-d-thiogalactopyranoside
kb	kilobase
kDa	kilodalton
K_D^{rel}	relative dissociation constant
l	Litre
LB	Luria Bertani
L-HBsAg	large surface antigen
M	Molar
mAb	monoclonal antibody
mg	milligram (10^{-3} g)
$MgCl_2$	magnesium chloride
M-HBsAg	medium surface antigen
min	Minute
ml	millilitre (10^{-3} l)
mm	milimeter (10^{-3} m)
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NDV	Newcastle disease virus
NK	natural killer
nM	nanomolar (10^{-9} M)
nm	nanometer (10^{-9} m)
nt(s)	nucleotide(s)
N-terminus	amino terminus
OD	optical density

ORF	open reading frame
P	polymerase protein
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PCR	Polymerase Chain Reaction
PEG	polyethylene glycol
pfu	plaque forming unit
pgRNA	pregenomic RNA
preC	precore
preC/C	precore and core
preS	hepatitis B preS genes
preS/S	preS and surface
PreS1	N-terminal region of L-HBsAg comprising 108 or 119 amino acid
PreS2	region of M and L-HBsAg comprising 55 amino acid
RF	replicative form
RNA	ribonucleic acid
RNase	ribonuclease
rpm	revolutions per minute
RT	room temperature
s	second
SDS	sodium dodecyl sulphate
S-HBsAg	small surface antigen
ssDNA	single stranded DNA
TAE	tris acetate EDTA buffer

Taq	thermus aquaticus thermostable DNA
TBE	tris-buffered EDTA solution
TBS	tris-buffered saline
TE	tris-EDTA buffer
TEMED	tetramethyl ethylenediamine
Th1	T helper 1
Th2	T helper 2
TP	terminal protein
USA	United States of America
v	Volt
v/v	volume/volume
WHO	World Health Organization
w/v	weight/volume
x *g*	centrifugal force
X-gal	5-bromo-4-chloro-3-indol-β-D-galactosidase

AMINO ACID ABBREVIATIONS

	One letter code	Three letter code
Alanine	A	Ala
Arginine	R	Arg
Asparagine	N	Asn
Aspartic acid	D	Asp
Cysteine	C	Cys
Glutamic acid	E	Glu
Glutamine	Q	Gln
Glycine	G	Gly
Histidine	H	His
Isoleucine	I	Ile
Leucine	L	Leu
Lysine	K	Lys
Methionine	M	Met
Phenylalanine	F	Phe
Proline	P	Pro
Serine	S	Ser
Threonine	T	Thr
Tryptophan	W	Trp
Tyrosine	Y	Tyr
Valine	V	Val