# Production of monoclonal antibodies for the detection of *Plasmodium vivax* lactate dehydrogenase

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# INTRODUCTION

- Malaria is a infectious disease by *Plasmodium* spp.
- . Plasmodium vivax
- . Plasmodium faciparum
- . Plasmodium malariae
- . Plasmodium ovale
- Of the four most common species that infect human, Plasmodium vivax and
  - P. falciparum account for 95% of infections.
- *Plasmodium* species also have the widest distribution, extending throughout the tropics, subtropics, and temperate zones.
- More than 300 million individuals throughout the world are infected with malaria, and more than 1 million people a year, most of whom are children, are being killed by this disease.



## INTRODUCTION



The life cycle of Plasmodium

## INTRODUCTION



The three stages of an attack of malaria

- The monoclonal antibodies (McAbs) have been used widely for diagnosis.
- Actually, diagnostic kits against *P. falciparum* infections have 88 ~ 98% sensitivity.
- But, in case of *P. vivax* infections, diagnostic kits have 70 ~ 80% sensitivity, which is remains a problem.



• To develop the dipstick kit had high sensitivity, it is necessary to produce the monoclonal antibodies with high affinity. So, First of all the present study was undertaken to produce hybridoma cells to be used for generating monoclonal antibodies with high affinity and specificity against *P. vivax* lactate dehydrogenase (PvLDH).

## **MATERIAL & METHODS**

	MA						_	-TYPE	FLIER	VAFEE	AT-SN	NETTT	VCTCA
	10	20		30	40		50	LUICE	60	UTIDIII.	70	MALLA	80
Homo-ldhla-bc	MA							-TIKS	ELIKN	FALLE	AI-HH	NKISI	VGTGS 30
Homo-ldha-xm-	MSWIV FVVRA	SQRVS SQRVS	ELCLG M	ALCP POATR	IPING	IWLRT	PVSKM	ATVKS	ELIER	FPSEK	PV-HH	SKVSI	IGTGS 79
Homo-1dhB-bc0	MA							-TLKE	KLIAP	VAEEE	ATVPN	NKITV	VGVGÇ 31
Homo-1dhB-xm-	MA							-TLKE	KLIAP	VAEEE	ATVPN	NKITV	VGVGÇ 31
Homo-1dhC-xm-	MS							-TVKE	QLIER	LIEDD	EN-SC	CKTTT	VGTGA 30
Pv-1dh-469811	MA							- <b>P</b>			K	AKIVL	VGSGN 15
Homo-ldhC-xm-	MS							-TVKE	QLIER	LIEDD	EN-SÇ	CKITT	VGTGA 30
	VGMAC AISIL	LKGLA DELAL	VDVAE DI	KLKG EMMDL	QHGSL	FLSTF	k	IVSSK	DYSVI	ANSNI	VIVTA	GARQQ	EGET-
	90	100		110	120		130		140		150		160
Homo-1dhla-bc	VGVACAISIL	LKGLS DELVL	VDVDE GI	KLKG ETMDL	QHGSP	FMKME	N	IVSSK	DATAL	ANSNL	VIITA	GAROK	KGET- 10
Homo-1dha-xm-	VGMACAISTL	LKGLS DELVL	VDLDE DI	KLKG ETMDL	QHGSP	FTRMP	N	IVCSR	DYFVT	ANSNL	VIITA	GARQE	KGET- 15
Homo-1dhB-bc0	VGMAC AISIL	GKSLA DELVL	VDVLE DI	KLKG EMMDL	QHGSL	FLQTP	K	IVADE	DYSVI	ANSKI	VVVTA	GVRQQ	EGES- 10
Homo-1dhB-xm-	VGMACAISIL	GKSLA DELVL	VDVLE DI	KLKG EMMDL	QHGSL	FLOTP	k	IVADE	DYSVI	ANSKI	VVVTA	GVRQQ	EGES- 10
Homo-IdnC-xm-	VGMACAISIL	TRUTA DELVL	VDVAL DI	KIKG EMMDL	QHGSL	1515	K	TTSGK	DISVI	ANSRI	VIVIA	GARQQ	EGET- IL
Homo-ldhC-xm-	VGMAC AISIL	TKDTS DETAT	VDVAL DI	KLKG EMMDL	QHGSL	FTSTS	K	ITSGK	DYSVS	ANSRI	VIVIA	GARQC	EGET- 10
	R LNLVC	RNVAI FKLII	PAIVK Y	SPDCKIIVV	SNPVD	ILTYV	AWKLS	GLPKN	RVIGS	GCNLD	SARFR	YLIGE	KLGIH
	170	180	//	190	200		210		220		230		240
Nomo 1dh1a bc	RLDLVG	RNVSI FRIMI	FNITG Y	SPIK KLLIV	TNEVD	ILTYV	AWKLS	GFPKN	RVIGS	GCNLD	SARFR	YFIGG	RLGIN 10
Homo-1dha-xm-	R LNLVC	RNVAI FKIMI	SSIVC Y	SPHC KLIIV	SNPVD	ILTYV.	AWKLS	AFPKN	RIIGS	GCNLD	TARFR	FLIGQ	KLGIH 23
Homo-1dhB-bc0	R LNLVC	RNVNV FKFII	POIVK Y	SPDC IIIVV	SNPVD	ILTYV	TWKL.S	GLPKH	RVIGS	GCNLD	SARFR	YLMAE	KLGIH 18
Homo-1dhB-xm-	R LNLVC	RNVNV FKFII	POIVK Y	SPDC IIIVV	SNPVD	ILTYV	TWKLS	GLPKH	RVIGS	GCNLD	SARFR	YLMAE	KLGIH 18
Homo-1dhC-xm-	RLALVC	RNVAIMKSII	PAIVH YS	SPDC KILVV	SNPVD	ILTYI	VWKIS	GLPVT	RVIGS	GCNLD	SARFR	YLIGE	KLGVH 18
Pv-1dh-469811	KEWNR DDLLP	INNKI MIEIG	GHIKK NO	CPNA FIIVV	TNPVD	VMVQL	LHQHS	GVPKN	KIIGI	GGVLD	TSRLK	YYISQ	KLNVC 17
Homo-1dhC-xm-	RLALVC	RNVAIMKSII	PAIVH YS	SPDC KILVV	SNPVD	ILTYI	VWKIS	GLPVT	RVIGS	GCNLD	SARFR	YLIGE	KLGVH 18
flomo-ldhla-bc Homo-ldha-xm- Homo-ldhB-bc0 Homo-ldhB-xm- Homo-ldhC-xm- Fv-ldh-469811	PSSCH GWILG 250 SESCH GWILG PSSCH GWILG PSSCH GWILG PTSCH GWILG PTSCH GWILG PTSCH GWILG	EHGDS SVPVW 260 EHGDS SVPVW EHGDS SVPVW EHGDS SVAVW EHGDS SVAVW AHGNK MVLLK	SGVNV A SGVNI A SGVNI A SGVNI A SGVNV A SGVNV A RYITV G	SVPL KE-LN 270 SVPL KD-LN SVPL KD-LN SVSL QE-LN SVSL QE-LN SVAL KT-LN SIPL QEFIN	PKLGT 280 FDIGT SDIGT PEMGT PEMGT PKLGT NKLIS	DSDSE DKDPE DKDPE DNDSE DNDSE DSDKE DAELE	NWKNU 290 QWKNU NWKEU NWKEU HWKNI AI	HKQVV HKEVT HKMVV HKMVV HKQVI FDRTV	ESAYE 300 ATAYE ESAYE ESAYE QSAYE NTALE	IIKIK VIKIK VIKIK IIKMK	GYT 310 GYT G-YTG G-YTG G-YT ASPYV	SWAIG SWAIG NWAIG SWAIG SWAIG APA	LSVAE 320 22 LSVAE 30 LSVAE 25 LSVAE 25 LSVME 25 AAIIE 24
Homo-IdhC-xm-	PISCHGWIIG	EHGDS SVPLW	SGVNVA	GVAL KT-LN	PRIGT	DSDRE	HWKNI	HKQVI	QSAYE	TIKLK	GYT	SWALG	LSVML 25
	19621 16016	SAU STURN	GUIGI EI	350	360	94194	370	NOBBE	380	SAUTE	390	DR-TP	400
Homo-1dh1a-bc								ENVHR	KK		350		200 23
Homo-ldha-xm-	LTESI LKNLR	RIHPVSTIIK	GLYGI DI	EEVF LSIPC	ILGEN	GITNL	IKIKL	TPEEE	AHLKK	SAKTT	WEION	KL-KL	38
Homo-ldhB-bc0	LIESM LKNLS	RIHPV STMVK	GMYGI EN	NEVE LSLPC	TLNAR	GLTSV	INOKL	KDDEV	AOLKK	SADTL	WDIOK	DLKDL	33
Homo-ldhB-xm-	LIESM LKNLS	RIHPV STMVK	GMYGI EI	NEVE LSLPC	ILNAR	GLTSV	INQKL	KDDEV	AQLKK	SADTL	WDIOK	DLKDL	33
Homo-1dhC-xm-	LVGSI LKNLR	RVHPV STMVK	GLYGI K	SELF LSIPC	VLGRN	GVSDV	VKINL	NSEEE	ALFKK	SAETL	WNIGK	DL-IF	33
Pv-1dh-469811	MAESY LKDLK	KVLIC STLLE	GQYG- HS	SDIF GGTPV	VICAN	GVEQV	IELQL	NSEEK	AK				FDEAI 30
Homo-1dhC-xm-	LVGSI LKNLR	RVHPV STMVK	GLYGI K	EELF LSIPC	VLGRN	GVSDV	VKINI	NSEEE	ALFKK	SAETL	WNIQK	DL-IF	33
		0											
	410												
Homo-ldh1a-bc													
Homo-ldha-xm-													
Homo-1dhB-bc0													
Homo-ldhB-xm-													
Homo-1dhC-xm-													
Pf-1dh-469811	AETKR MKALA	8											31

Amino acid sequences alignment of lactate dehydrogenases (LDH) from *P. vivax* and *Homo sapiens* isoforms. Each antigens were produced three specific region of *P. vivax* LDH, #1, #2 and #3, respectively.

# Amino acids sequence of recombinant polypeptides specific for *P. vivax* LDH.

Fractions	Amino acids sequence					
# 1	-SKAKIVLVGSGMIGGVMATLIVGKNL-	26 amino acids				
# 2	-FTKAPGKSDKEWNRDDLLPLNNKIMIEIGGH-	31 amino acids				
# 3	-DYG-HSDIFGGTPVVLGAN-	18 amino acids				

## Hybridoma technique



- A. Immunization of mice
- B. Cell fusion of myeloma cells and spleenocytes
- C. Production of hybridoma cells
- C. ELISA screening
- D. McAbs cloning & determination of McAbs isotype
- E. Purification of McAbs from mouse ascites
- F. Sensitivity test and patient blood test by ELISA



#### **SDS-PAGE** bands pattern



**SDS-PAGE bands pattern of the** *P. vivax* merozoites lysate. 15% gels were prepared, and lysate was electrophoresed under reducing conditions and stained with coomassie blue. The 33 kDa of band (arrow) indicate presumed *P. vivax* lactate dehydrogenase. P.v, *P. vivax* lysate; M, prestained molecular size marker.

## Antigen reactivity by ELISA



Antigen reactivity of the hybridoma cells culture supernatant. A405 values of PvLDH (n=3) were determinated by indirect ELISA. Normal serum was diluted 1:200. PBS was control.

## Antigen reactivity by ELISA



**Antigen reactivity of the ascitic fluids.** A405 values of PVLDH (n=3) were determinated by indirect ELISA. Normal serum was diluted 1:200. PBS was control.

## Western blot analysis



Western blot analysis of four McAbs reacted with the *P. vivax* merozoites lysate. *P. vivax* merozoite lysates were separated by SDS-PAGE and reacted with McAbs. Only one strong reaction band with 33 kDa molecular weight (arrow), persumed LDH, was detected. M, molecular size marker; Lane 1, B4D; Lane 2, C2B; Lane 3, D2H; Lane 4, D7E; Lane 5, normal mouse serum.

## Isotyping of monoclonal antibodies

• Mouse monoclonal antibody isotyping kit (Sigma)

	B4D	C2B	D2H	D7E
Ab Isotype class	lgG2b	lgG2b	lgG2b	lgG2b

# Antigen detection by indirect ELISA

(P. vivax patient's blood / PvLDH recombinant Ag)



Coating Ag: *P. vivax* patient's blood (100ul) / pvLDH recombinant Ag ( 2 ug/ml) Primary Ab: Ascites McAb D2H / D7E (1:500 dilution)

## Affinity test by direct ELISA

#### (McAb D2H and D7E - biotin conjugation)



Coating Ag: P. vivax patient's blood

Primary Ab: McAb D2H and D7E purified IgG conjugated with Biotin

# **IgG** purification

### P. vivax LDH monoclonal antibody

- Production of ascites fluid from BALB/c mice
- Antibody Purification (IgG)
  - : ImmunoPure (protein A) IgG purification Kit (Pierce)
- Antibody Conjugation (IgG-HRP conjugation)

	B4D	C2B	D2H	D7E		
Purified Ab	1.1 mg/ml	1.2 mg/ml	1.9 mg/ml	1.1 mg/ml		
Ab-HRP conjugation	1 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml		

### Sensitivity test by sandwich ELISA





### Sensitivity test by sandwich ELISA



## **CONCLUSIONS**

- We undertook to produce hybridomas generating McAbs of *P. vivax* lactate dehydrogenase (PvLDH).
- Four McAbs (B4D, C2B, D2H, D7E) were obtained.
- The isotypes of all McAbs were IgG2b type.
- Purified antibodies were tested with HRP-conjugated antibodies, and bloods of *P. vivax* patients by sandwich ELISA, showing the 98% sensitivity.