

# Study of Hepatitis B Virus Desoxyribonucleic Acid in Blood Donors with Negative Hepatitis B Surface Antigen and Positive Anti Hepatitis B Surface

Dwi Yulia<sup>1</sup>, Ellyza Nasrul<sup>2</sup>, Nasrul Zubir<sup>3</sup>, Yanwirasti<sup>4</sup>

Medical Faculty, Andalas University, Padang, Indonesia

**Abstract:** *Infection of HBV is still one of the highest burden disease in the world. It is estimated that two billions of the worlds populations has had contact HBV. HV related complications such as cirrhosis and hepatocellular carcinoma (HCC). One of the contamination of HBV was transfusion of blood that infected by HBV. The aim of study to determinmine there is HBV DNA at blood donors with negative Hepatitis B surface antigen negatif and positive anti hepatitis B surface. Desain of this study is deskriptive cross sectional. The study was conducted at clinical laboratory, UTD RSUD Prof DR MA Hanafiah SM Batusangkar and Sawahlunto and Biomedical Laboratory, Faculty of Medicine, Andalas University. From Oktober 2014 to March 2016. Specimen of this study are blood donors with negative Hepatitis B surface antigen and positive anti hepatitis B surface that tested using strip by kromatografi method. HBV DNA was tested by konventional PCR and occult B Hepatitis Infection by RT PCR. The sekuensing of nukleotida was sent to Macrogene, Korea. The result of this study is there is no HBV DNA at blood donors and there is no occult B Hepatitis B at blood donors with negative HBsAg and positive anti HBs. This test using kontrol positif that estimated by sekuensing nukleotide of HBV. The conclusion of this study is there is no HBV DNA and occult B Hepatitis at blood donors with negative HBsAg and positive anti HBs. To suggest for using anti HBs test at blood donors with negative HBsAg to stoped transmsion of HBV. Infection of Hepatitis B Virus is still one of the highest burden disease in the world. It is estimated that two billions of the worlds populations has had contact Hepatitis B Virus. Hepatitis B Virus related complications such as cirrhosis and carcinoma. One of the contamination of Hepatitis B Virus was blood donors that infected by Hepatitis B Virus. The aim of study to determine there is Hepatitis B Virus DNA at blood donors with negative Hepatitis B surface antigen negative and positive anti hepatitis B surface. Design of this study is cross sectional. The study was conducted at clinical laboratory, Unit blood donors in hospital and Biomedical Laboratory, Faculty of Medicine, West Sumatra, From October 2014 to March 2016. Specimen of this study are blood donors with negative Hepatitis B surface antigen and positive anti hepatitis B surface that tested using test strip. method. Hepatitis B Virus DNA was tested by conventional Polymerase Chain Reaction and occult B Hepatitis Infection by RT Polymerase Chain Reaction. The result of this study is there is no Hepatitis B Virus DNA at blood donors and there is no occult B Hepatitis B at blood donors with negative Hepatitis B surface Antigen and positive anti Hepatitis B surface. The conclusion of this study is there is no Hepatitis B Virus DNA and occult B Hepatitis at blood donors with negative Hepatitis B surface Antigen and positive anti Hepatitis B surface.*

**Keywords:** HBV DNA , HBsAg, Anti HBs, PCR, RT-PCR, Blood donors

## 1. Introduction

### 1.1 Background

Infection of Hepatitis B represent the problem of especial health in the world. More than two billion of the world population have been infected by Virus of Hepatitis B (VHB). It is estimated that 400 - 450 million among them suffer from hepatitis B which later on can suffer chronic hepatitis B, liver cirrhosis or liver cancer. 65 million death from infected B hepatitis patients were resulted by cirrhosis or carcinoma hepatoselular ( Aspinal, 2011; Zubir, 2013; Oakes, 2014).

Infection prevalence of hepatitis B chronic varies in some places in the world, and infected people age is also different one another. Highest prevalence, 8-15% is in South-East Asia, African, Archipelago Of Pacific, Amazon of Basin. In East America, North and South, infection attack more than 60% moppet child. The lowest prevalence, less than 2%, are in Europe population, where infection of this VHB is more in adult group infection. Indonesia occupies the third place to the amount of patient of hepatitis B, after China and India. Hygienist of Division of Herpetology Departmental Disease In University Indonesia, Sulaiman in his research in 2000 mentions that more or less 13 million residents of Indonesia are infected by HBV. Till now there is no report concerning

occurrence of Hepatitis B in Indonesia, existing newly data at health public Center (RSUP) from various metropolis, like research of Yulius and of Hanif in RSUP DR M Djamil in the year 1973, in the reality infection of VHB was found at the age 12-30 year with occurrence differ between woman and men ( Hadi, 2002)

Research concerning HBV is also conducted by Zubir and Siburian in ethnic of Minangkabau (2011), epidemiology molecular, by collecting sample of Red Cross Indonesia Branch Field and from some patient taken care of Hepatitis B chronic in RSUP DR.M.DJAMIL Field, obtained by VHB C genotype is more dominant found in ethnic of Minang, followed by VHB B genotype and mixture of genotype B and C. In the year 2013, Zubir and of Siburian again conducted research of VHB in Minangkabau ethnic, reported that by using primary mix, found VHB of C genotype which its mutation at Pre-S, and dropsy of core promoter ( BCP).

Virus of Hepatitis B is virus of double- stranded Desoxyribonucleic acid ( DNA) in the form of circular and including Hepadnaviridae family. VHB multiply themselves through RNA transcriptase reverse, needing enzyme of reverse transcriptase for the synthesis of nucleotide. Hepadnavirus have been recognized since last 19 thousand year and have experienced of at least evolution 7,72 x 10 substitution/location/year. Morphology of HBV globular

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with double shroud, lapped over external shroud especially by Hepatitis B Antigen surface (HBSAG), while shroud inside which is called nucleocapsid of core lapped over of Hepatitis B antigen core (HBCAG), there are HBV DNA and enzyme of polymerase which good for virus replication (Zubir, 2013; Ismail, 2014)

Virus of Hepatitis B can attack all age, start from baby, adolescent, adult to old age. This Hepatitis B can be in the form of asymptomatic, infection of hepatitis B chronic, and long-range can cause damage of hard liver like ossification of cirrhosis or liver (2.4-3.5% /year) liver cancer or carcinoma hepatoselular (3-6% /year) which tip of [at] death (Vivekanandan, 2010; Aspinall, 2011)

Infection of HBV can pass through the body that contain dilution or blood [of] VHB. Infection can be in form of horizontal transmission like sexual contact which is not protected, blood transfusion, usage of needle which is contaminated. There are VHB in low concentration in cement, vagina secret, tear, sweat, mother milk water and urine. Other infection can be vertically, that is from mother to child during delivery process. Beside that VHB can infected between family member in household, it can be in the form of chafed husk contact, or membrane of mucosa with VHB (Valsamakis, 2007; Paganelli, 2012)

Infection of Hepatitis B give symptom of clinics typical and vary, that is body felt to weaken, pain in bone at right stomach, queasy, puking, chromatic urine water can be condensed tea, eye and entire body can turn to yellow (Hadi, 2002).

Diagnosis of VHB can be done with laboratory check up, that is serology in the form of check up Hepatitis B Antigen surface (HBSAG) provided many commercially, inspection of Hepatitis B Antigen core (HBCAG), Hepatitis B e Antigen (HBeAg), Anti HBc, Anti HBs, which is done by ELISA, come up with inspection of DNA VHB, virus genotype, sub genotype and inspection of virus structure by molecular like estimated to HBV X Gene sliver its relation with HCC (Kew, 2011). Inspection of serology for VHB with available test rapid till now is HBSAG and Anti HBS, while for the inspection of other serology need special appliance and skillful energy to do inspection.

Unit of Transfusion Blood Hospital (UTDRS) is the place unit provides transfusion blood to hospital which is there is no closest UTD Red Cross Indonesia. UTDRS have role of alliance between UTD and Blood Bank Hospital (BDRS) with especial fundamental duty as follows: start donor selection, intake of donor blood, donor blood skirting from 5 disease, compatible crossed test, dissociation of blood component, serving request all clines', until giving of blood for transfusion to patient requiring blood, reporting and record-keeping and also reference conducted by in UTDRS (DEPKES RI, 2008).

Service of blood transfusion started by conducting donor candidate recruitment, that is collecting people who are ready to become blood donor. Here in after select donor to get voluntary donor at risk to lowering, to be continued with inspection of blood group and hemoglobin. After process

intake of donor blood, taken again blood sample for the reexamining of blood group of ABO, test and rhesus filter, that is inspection to disease of Catching Infection Pass Transfusion Blood (ILMTD) for example: Syphilis, Hepatitis B, Hepatitis C, and HIV. For area with its high malaria prevalence can be enhanced with inspection of blood malaria (Health Department, 2008).

Inspection of VHB in Blood Transfusion Unit at home Pain (UTDRS) till now still use HBSAG strip test, because beside its cheap price, it can be done without specialty. Strip HBSAG distributed by Health Department every year as according to requirement of UTDRS. But this inspection have weakness, the inspection cannot detect virus which is rate of HBV its DNA < 104 coffee, so that people with HBV at phase early infection with virus rate which still lower or patient of HBV silent, cannot detect with inspection of HBSAG strip test. In tidiness do not be explained by antibody of monoclonal type. By molecular, part of VHB surface have some type of antigen. Patient of VHB with existence of mutation at part of VHB surface, of course cannot detect by using HBSAG strip test.

To diagnose the inspection of HBSAG in general use antibody addressed for epitaph found on determinant -a. conformational Epitaph of determinant-a stabilized with a backbone which consist of residue which tied by disulfide. Sour sequence amino at HBSAG have very domain and hydrophilic of conformational and lay in sequence 100-160 and known as is fortunate of loop hydrophilic of HBSAG. Especial Antigenic Determinant (a-determinant) represent area of imuno dominant of HBSAG which lay between acid of amino 121 and 147 in is fortunate of loop hydrophilic. A Determinant HBV wild type lay in a 140-146. Change of this epitaph conformational cause failure of antibody for the neutralize of infection of VHB and degrade reagent for the inspection of HBSAG (Valsamakis, 2007).

Virus of Hepatitis B have it unique, that is there are stadium of RNA needing mechanism of transcription return to become form of DNA accompanied by mutation form. Other characteristic is ability to maintain infection in the form of DNA circular closed covalently (cccDNA) which remain to in core of cell of hepar as printing; mould for the forming of new virus. Particle of Dane or of virion intact HBV in number 107 - 109 virion / patient serum mL, at this condition, if inspection to HBV with just HBSAG, can be found in result of spurious negativity. Natural tend to be VHB mutation because it does not have corrective system of moment of replication. This matter of inexistence cause 3',5'exonuclease to correct mistake of moment nucleotide insersi of transcription return (Zubir, 2013).

Various clinic study and epidemiology report there are four pattern of mutant VHB, that is gene mutant of S, and pre core of core gene promoter of C [at] gene of X, and gene P. Gene Mutant of S happened at Pre-S1 regio, Pre-S2, and regio S. mutation of Regio Pre-S and of Pre-S2 cause change of HBSAG synthesis promoter, so that produce HBSAG decrease. This matter cause HBSAG react negativity at inspection of serology. Mutation of Regio S is more regular reported and have broader implication, specially when it happened at gene penyandi of determinant-

a which have potency to generate failure detect VHB donor blood and patient, because available medium unable to detect HBSAG which have mutation, second is failure of vaccination, because antibody result of vaccination cannot neutralize VHB which have mutation (mutant escape). One of the form of mutant met mutation of G become acid of amino 145 (G145R), is often reported at case failure of vaccination (Muljono, 2007).

Research at patient accepting transfusion was conducted by Liu et al. (2010) in China. It was Found 5 from 2972 (0,13%) receiver of blood suffer hepatitis occult, in the reality by filo genetic, found by relation which close to donor blood which also suffer hepatitis occult.

Research in China South-East by Yuan (2010) mentioned that there is occult HBV at donor blood, after checked by ITS. HBV, where with inspection of HBSAG do not detect, this Occult HBV is attributed to be found by HBV mutant and existence of emphasis of virus replication and gene expression and low virus (Yuan, 2010).

Similar research was also done in Bangladesh by Mahtab (2012), by finding DNA VHB, while inspection with HBSAG found negativity. Detected at 8 from 20 patient with liver cirrhosis cryptogenic, 2 from 10 patient with carcinoma hepatocellular, and 2 from 10 subject with rate of ala nine aminotrasferase which mounting.

Detecting donor blood by Bag (2012) in Turki also find inspection of negative HBSAG, but after it was conducted] inspection of HBV DNA by using reaction chain polymerase (PCR), its result; from 401 negative HBSAG, in the reality 45 people have anti positive HBC, with PCR found 3 from 45 donor blood of DNA VHB. The research of this Bag, suggested donor blood with needed negative HBSAG ] inspection Anti HBC beforehand, where necessary conducted by inspection of HBV DNA, correct donor blood free from HBV.

Research of donor blood with negative HBSAG was also conducted in Egypt by Badrawy (2013), in the reality 2 from 7 donor blood with anti positive HBC, its positive DNA VHB. Become to be suggested the importance of inspection of HBV DNA [at] donor blood which anti positive HBC, more than anything else if titer anti Its [of] low him.

Research by Paganelli (2012) found that after infection by HBV, antibody to HBV surface (Anti HBS) formed sixth in infection pasca or at a period of cure. But ] other research mention with formed anti HBS, there are some possibility, among others patient recover perfection, both HBV there is still but low in number or HBV occult, hereinafter [is] HBV super infection with HBV mutant. The condition above have potency for the infection of HBV, especially at patient accepting transfusion blood, also can be catching to officer of laboring laboratory [do] not follow order, so that number occurrence of Hepatitis B progressively mount.

Research a donor blood have been done by Theja (2010) Institute of Eijkman, Mahtab (2012) in Bangladesh, Badrawy (2012) in Turki, in the reality with donor blood

with negative HBSAG, found DNA VHB . Existence of DNA

VHB at research above connected to sliver with inspection Anti HBC which are positive. Inspection Anti HBC need special appliance and skillful energy to do so, whereas inspection of available test rapid till now only HBSAG and Anti HBS.

Pursuant to the things above, hence require to be done by research about inspection of DNA virus of hepatitis B at donor blood with negative B antigen surface hepatitis and anti positive B surface hepatitis with a purpose to so that correct donor blood free from VHB

## 1.2 Formulation of the problem

Based on the explanation in background above, it can be formulated the problem of research as follow: 1. Is there any DNA VHB at donor blood with negative HBSAG and positive anti-HBs 2. Is there Hepatitis B occult at donor blood with negative HBSAG and Anti HBS'POSITIF?

## 1.3 The Purpose of Research

Target of public:

Studying Virus acid desoxyribonucleic of Hepatitis B at donor blood with negative antigen surface B hepatitis and anti positive surface B hepatitis

Special Target 1. To study inspection of DNA VHB at donor blood with HBSAG negative and anti positive HBS 2. To study inspection of B hepatitis occult at donor blood with Negative HBsAg and Anti HBS'POSITIF.

## 1.4 The Benefit of research

1.4.1 Science 1. This research can give contribution to the development of science inspection of DNA VHB at donor blood with negative HBSAG and Anti HBS'POSITIF

### 1.4.2 Practitioner

By finding this research can make base formulation of policy of national, so that donor blood skring especially for VHB will be conducted by molecular, and blood skring which pursuant to re-studied just HBSAG to importance of society. Furthermore, this implication become valuable information to clinics, so taking a care in giving blood transfusion or blood product at patient.

1.4.3 Benefit to society 1. Degrading painfulness number and death of effect of HBV, and also open preventive possibility of infection because HBV through blood transfusion.

## 2. Method of Research

### 2.1 Type and design of research

The research is descriptive sectional cross (study exploratory) with aim for the inspection of DNA Virus of Hepatitis B (VHB) in donor blood with HBSAG(-) and Anti HBs (+)

## 2.2 Population, sample, and sample technique

### 2.2.1 Population

Population in this research is all donor blood with negative HBSAG and Anti positive HBS in UTD RSUD Prof.Dr.Ali Hanafiah SM Batusangkar and UTD RSUD Sawahlunto.

### 2.2.2 Research Sample

Research Sample is the part of population fulfilling criterion of inclusion, that is donor blood plasma with negative HBSAG and Anti positive HBS. Criterion of Exclusion a. Blood of Lyses b. History immunize VHB

### 2.2.3 The amount of sample

Amount of sample in this research is specified pursuant to formula, with value of  $\alpha = 0,05$

$$n = z^2 \frac{PQ}{d^2} \quad n = 202$$

$$d = 2$$

$n$  = Amount of minimum sample

$Z$  = normal standard deviate to = 0,05, hence  $z = 1,96$

$P$  = Proportion of Hepatitis B ( data of WHO year 2013  $\pm 5$

$$Q = 1 - P$$

$d$  = used precise 3

### 2.2.4 Technique intake of sample

Technique intake of sample in this research is non sampling random, that is sampling consecutive, where incoming subject and fulfill election criterion packed into research until the amount of needed to subject fulfilled. Plasma kept at temperature - 200C in Laboratory Pathology Clinic until inspection time done.

### 2.2.5 The sample amount

The sample Amount in this research is formula to pursuant specified,  $\alpha$  of value with = 0,05

$$n = z^2 \frac{PQ}{d^2} \quad n = 202$$

$$d = 2$$

$n$  = Of minimum Amount of sample

$Z$  = normal [of] standard of deviate to = 0,05,  $z$  hence = 1,96

$P$  = Proportion Of Hepatitis B ( data of WHO year 2013  $\pm 5$

$$Q = 1 - P$$

$d$  = precise used 3

### 2.2.6 Technique of taking sample

In this research, sample technique is non sampling of random, that is consecutive sampling where coming subjects that fulfill criterion election. The plasma was Kept at temperature - 200C in Laboratory Pathology Clinic time inspection.

## 3. Operational Definition

DNA HBV

Definition: Inspection of acid of nucleate [at] circuit of VHB in blood

Measuring instrument: PCR qualitative use gel method of electrophoresis

Way of measure: comparing ribbon of DNA formed with marker

Result of measure: positive and negative

Measure scale: nominal 4. Occult Hepatitis B

Definition: Inspection of acid contents of nucleate [at] circuit of VHB

Measuring instrument: RT-PCR

Way of measure: comparing curve of DNA formed with marker

Result of measure: height of formed curve

Scale measure: ratio

## 4. Materials and Research Instrument

Materials and Instrument which is required in this research cover donor blood plasma for the insulation of DNA virus of Hepatitis B, instrument and materials for the inspection of DNA VHB, instrument and materials for the PCR OF and identified sequence

### 4.1 Research Materials

Blood plasma Donor with result of inspection of negative HBSAG and positive Anti-Hbs, done by of insulation of DNA Virus of Hepatitis B. then inspection of DNA HBV use PCR qualitative use gel method of electrophoresis. Result of DNA HBV which is positive, the inspection continuation in the form of RT PCR to determine whether there is Hepatitis occult B.

### 4.2. Research Instrument

#### Collecting sample

Sample was collected by dissociating plasma of donor blood with negative HBSAG and Anti positive HBS. Sample made by aliquot and kept at temperature - 200C.

Extraction and of amplification of DNA VHB:

DNA VHB extract from 200 of Sample plasma L use mini kit QIAMP DNA blood ( Qiagen, Hilden, Germany). Working procedure as according to guide released by fabric,(work procedure is enclose), later; then 80  $\mu$  Eluted DNA L kept at temperature - 80 0C by inspection of PCR

Working procedure of PCR gradient / conventional

A. Procedure making of PCR mix master with primary of P& and P8 1. Enhancing 12,5  $\mu$  L Gotaq green into PCR tube 0,2 mL 2. Enhancing primary of P7 counted 1,5  $\mu$  L in PCR tube 0,2 mL 3. Enhancing primary of P8 counted 1,5  $\mu$  L in PCR tube 0,2 mL 4. Enhancing Nuclease Free Water counted 6,5  $\mu$  L into PCR tube 0,2 mL 5. Enhancing DNA sample counted 3  $\mu$  L into PCR tube 0,2 mL 6. Mix by using vortex, later; then included into machine of PCR

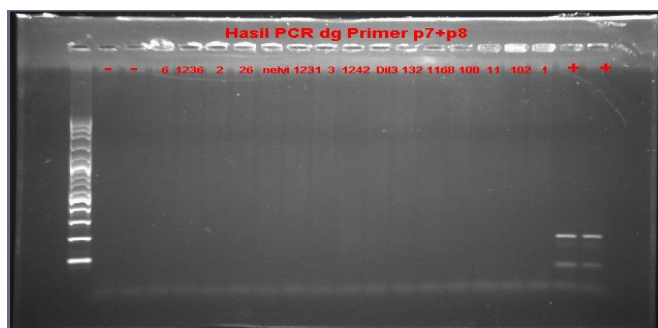
B. Program of PCR for the primary of P7 and of P8

Input Data of PCR the following 1. Denatures Pre : 94 0C during 3 minute 2. Denatures : 94 0C during 1 minute 3. Annealing: 65 0C during 1 minute 4. Extension : 72 0C during 2 minute 5. Repeat process of denatures, and annealing of extension counted 39 cycle 6. final of Extension : 72 0C during 7 minute 7. Down Cold : 12 0C during 10 minute 8. Program example [of] of PCR





C. Procedure of electrophoresis result of PCR P7 and of P8  
 1. Deliberate 1,5 gram of agarose, then put into bottle media. Enhancing 100 TE mL 0,5 X and Gel of Red counted 3 µ L, shake homogeneous till. Heating in microwave till boil 2. Then included into printing; mould and wait till gel of agarose hard 3. Enhancing 10 µ L result of PCR with primary of P7 and of P8 into well and last enhance 3 µ L Ladder 100 bp 4. Running with voltage 120 volt during 1 hour 5. After finishing dielectroforesis, see the result of doc below



D. Procedure of PCR with primary of HBS1 and of HBS2  
 1. Result of product of PCR with primary of P7 and P8 taken 1 µ L, then dissolved into 100 µ L Nuclease Free Water 2. Enhancing 12,5 µ L Gotaq Green into PCR tube 0,2 mL 3. Enhancing primary of HBS1 counted 1,5 µ L into PCR tube 0,2 mL 4. Enhancing primary of HBS2 counted 1,5 µ L into PCR tube 0,2 mL 5. Enhancing Nuclease Free Water counted 8,5 µ L into PCR tube 0,2 mL 6. Enhancing result of PCR which have been thinned mentioned counted 1 µ L into PCR 0,2 mL 7. Mix by using vortex, then put into machine of PCR

E. Procedure of electrophoresis result of HBS1 and of HBS2  
 1. Deliberate 1,5 gram of agarose, then put into bottle media. Enhancing 100 TE mL 0,5 X and Gel of Red counted 3 µ L, shake homogeneous till. Heating in microwave till boil 2. including into printing; mould and wait till gel of agarose hard 3. Enhancing 10 µ L result of PCR with primary of HBS1 and of HBS2 into well and last enhance 3 µ L Ladder 100 bp 4. Running with voltage 120 volt during 1 hour 5. After finishing dielectroforesis see result of gel doc below



Scheme work PCR gradien can be seen as follow  
 DNA

PCR with primary of P7 and of P8

Result of PCR P7 and of P8 dielectroforesis

Result of electroforesis seen in gel of doc

Result of PCR with primary of P7 and P8 in PCR with primary of HBS1 and of HBS2

Result of PCR P7 and P8 in PCR with primary of HBS1 and of HBS2 dielectroforesis

Result of electroforesis is seen in gel doc

Result of PCR HBS1 and HBS2 in data of Sequence processing

Picture 4.2. Scheme work PCR gradien

### 4.3 Working procedure of Real Time PCR

A. Procedure Making of Master Mix for the Real Time PCR

1. Result of product PCR with primary P7 and P8 counted 1 µ L, then dissolved into 100 µ L Nuclease Free Water 2. Enhancing 5 µ L Eva green into Real Time PCR tube 3. Enhancing 3 µ L Nuclease Free Water into Real Time PCR tube 4. Enhancing 0,5 µ L Primary of HBS1 into Real Time PCR tube 5. Enhancing 0,5 µ L Primary of HBS2 into Real Time PCR tube 6. Enhancing 1 µ L result of PCR with primary of P7 and of P8 which have been thinned previously ( see no.1) into Real Time PCR tube

B. Program of Real Time PCR with primary of HBS1 and of HBS2

Input Data program of the following Time PCR 1. Activation Enzyme : 98 0 C during 2 minute 2. Denatures : 98 0 C during 5 second 3. Annealing : 9650 C during 5 second 4. Repeat process of denatures, and annealing of extension counted 39kali 5. Curve Melt : 65-95 during 5 second

Following program example [of]:



As for problem of ethic which possible arise : Subject HBV DNA` will be informed that it shall] no longer, to prevent infection of HBV to individual accepting the blood

#### 4.8 Data processing

Data which have been gathered will be presented in the form of diagram and tables.

### 5. Result of Research

Research of characterization, inspection of DNA VHB have passed some steps. First phase was intake of sample in UTD RSUD Prof.Dr MA Hanafiah SM and RSUD Sawahlunto. Sample ws collected 375 sample which have been checked by its HBSAG use HBSAG strip test, and expressed negativity. Sample was taken through blood sack; bag hose

and put into sterile place. The phase was done by inspection anti HBS by using strip test. Sample with anti HBS which are positive dissociated and kept temperature - 200C

#### 5.1. Characteristic of Research Subject

From 375 sample with result of negative inspection HBSAG, then the inspection was done for anti HBS. After the inspection, it was found 238 donor blood sample with negative HBSAG and anti positive HBS. All subjects were given code according to sequence and code of checked sample, taken away from 2 UTD RS, that is UTD RSUD Prof.Dr MA Hanafiah SM and UTD RSUD Sawahlunto. Characteristic of Subject research can be seen tables 5.1 hereunder.

**Table 5.1:** Characteristic of Subject Research

No	characteristic	Hospital Prof.DR MA Hanafiah SM		Hospital Sawahlunto		inspection HBSAg	Inspection anti HBS
		f	%	f	%		
1	Age					negatif	positif
	20-30	25	19,53	7	6,36		
	31-40	60	46,88	56	50,91		
	41-50	38	29,69	40	36,36		
	> 50	5	3,91	7	6,37		
2	gender					negatif	positif
	male	112	87,50	102	92,73		
	female	16	12,50	8	7,27		
Total		128		110			

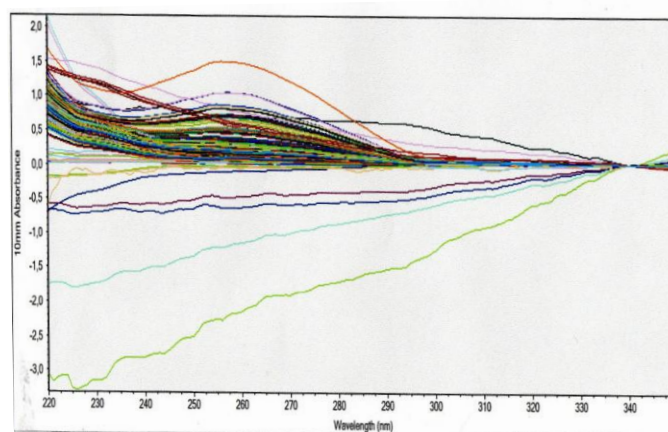
from tables 5.1 above, it can be seen that blood donor residing in many gyration old age 31-40 year, and compared to more man proportion of woman. Result of inspection all subject for HBSAG is negative and anti HBS is positive.

#### 5.2 DNA VHB Blood Donor with Negative HBSAG and positive Anti HBs

##### 5.2.1 Insulation DNA Virus of Hepatitis B

In this research, 238 samples were then conducted by insulation of DNA VHB. Insulation was done according to procedure which have been specified by reagen kit. DNA VHB destruction from 200 µ Serum L / plasma by using mini kit reagen QIAMP DNA blood ( Qiagen, Hilden, Germany) according to instruction specified by factory

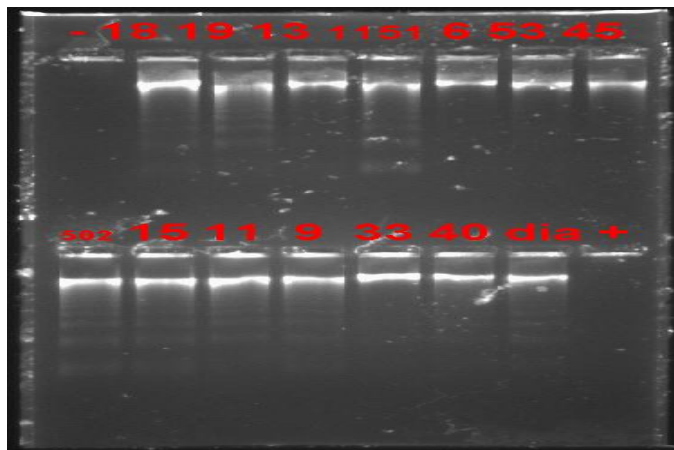
DNA which had destruction was under inspection of sour concentration nukleat at wavelength 260 nm use drops. From total sample above, the success DNA VHB of diekstraction with concentration of DNA vary one another, like seen at Picture 5.1.



**Picture 5.1:** Concentration of DNA VHB at wavelength 260 nm

In picture 5.1, it can be seen the result of concentration of DNA vary, from 0,5 ng / µ L - 34,6 ng / µ L. ( Result enclosed). Measurement of concentration of DNA use Nano drop 2000 spectrophotometer with parity 1,15-2,91. In this research, result of insulation DNA VHB is also dissociated by using gel of elektroforesis 1,2% in buffer 0,5x TBE. Result of insulation of DNA can be seen [by] [at] picture 5.2 hereunder.





**Picture 5.2:** Electroforesis DNA VHB

In picture 5.2 it can be seen the result of DNA VHB elektroforesis and can be explained that insulation of DNA was success VHB

### 5.2.2 Design primary for PCR

Pursuant to epidemiology data spreading of VHB in West Sumatra, hence chosen primary 4 kinds of specific primary (primary specification analyzed with BLAST

**Tables 5.2:** Analysis and Sequence of specific primary for the nested PCR DNA HBV

No	Primer	Sequence primer 5'-3'	Polarities	Position nukleotida
1	P7	5'-GTCGTCGACTTCTCTCAATTTTC-3'	S	256-278
2	P8	5'-CGGTAWAAAGGGACTCAMGAT-3'	AS	796-776
3	HBS1	5'-CAAGGTATGTTGCCCGTTTG-3'	S	455-474
4	HBS2	5'-AAAGCCCTGCGAACCCTGA-3'	AS	713-694

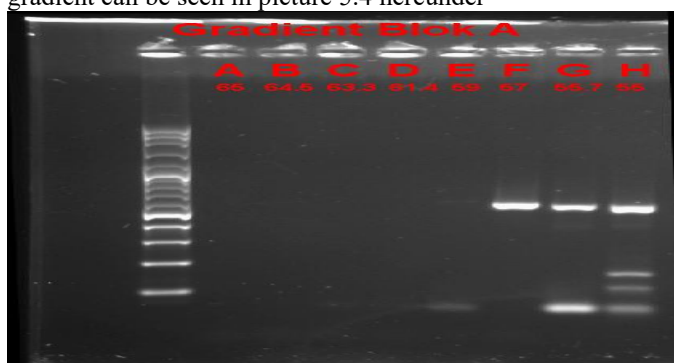
S = ACE sense= Antisense

Existence of variation spreading of VHB in Indonesia, specially West Sumatra, it was chosen primary for amplification mechanically PCR use method of nested PCR, that is using primary outer of P7 and P8, for the PCR OF first phase and primary inner of HBS1 and HBS2 for the PCR of second phase.

### 5.2.3 Result of conventional PCR for positive control

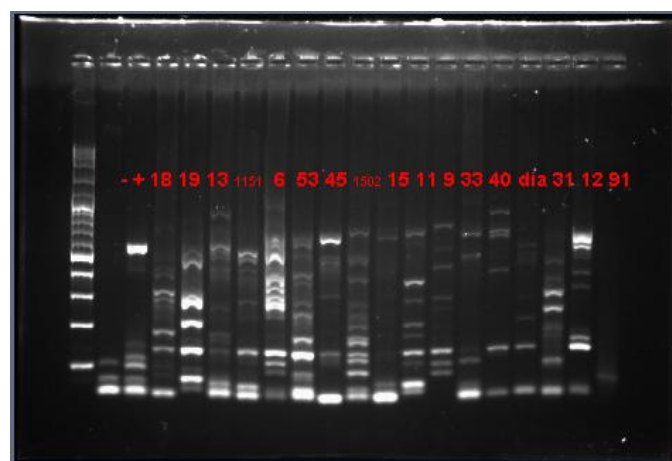
Result of primary optimatation for the nested of PCR use P7 and P8 at first phase for positive control obtained at temperature 650C, continued with HBS1 and HBS2 for second phase

Pursuant to guide of research using primary above, temperature of annealing for the primary of the primary reside in temperature 550 C. But in this research, stipulating of temperature 550 the C at elektroforesis do not be obtained by single ribbon, but many ribbons bothering result of interpretation. It hereinafter tried with temperature variation between 550C until 650C. Result of elektroforesis at temperature under 650C cannot be obtained by single ribbon, but there are additional ribbon which bother product although interpretation of PCR yielded very specific. Result of PCR for positive control with different temperature gradient can be seen in picture 5.4 hereunder



**Picture 5.4:** Result of positive PCR control with temperature 650C until 550C [at] phase of outer Primary

In Picture 5.4 above, it can be seen that by using primary phase of outer with temperature under 650 C, got some ribbon at its result, so that complicate for the interpretation of its result in this research, researcher also tried to do research at some sample with temperature 550 C, it was also found many ribbon in its result, and many sample giving positive result counterfeit. Result of inspection in some the sample can be seen in Picture 5.5 hereunder.



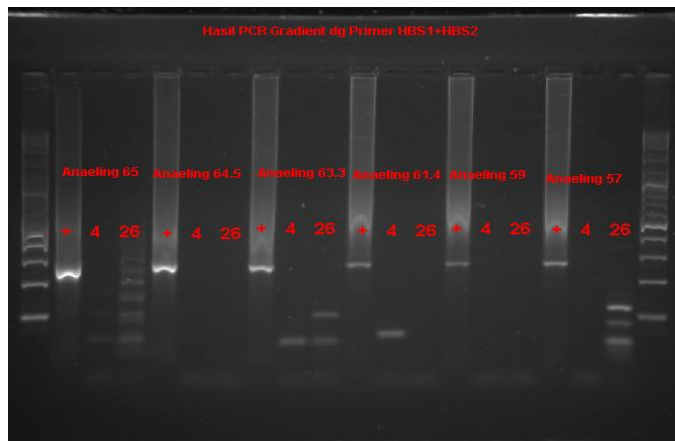
**Picture 5.5:** Result of PCR nested elektroforesis use primary of P7 and P8 at temperature 550C

In Picture 5.5 above, it can be seen to emerge some good ribbon in positive control and also in sample which is elektroforesis done. Pursuant to the result above, hence temperature of annealing which commonly use with primary of P& and P8 temperature 550 C cannot be conducted, and specified by using temperature 650 C for the phase of outer by using primary of P7 and P8

For second phase, by using method of nested PCR, using primary of HBS1 and HBS2, it was also conducted by some examination with temperature 55 0C - 65)C for its



temperature, result of positive control elektroforesis and some sample earn in seeing Picture 5.6 hereunder.

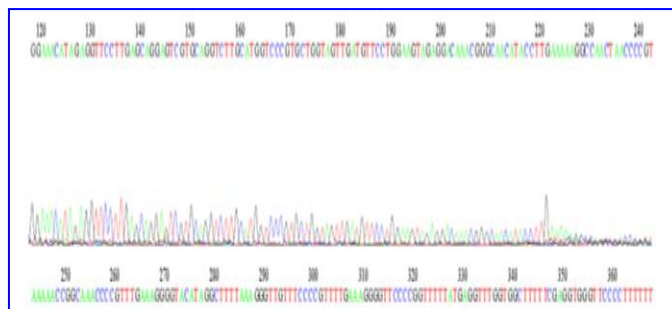


**Picture 5.6:** Result of positive PCR control primary inner HBS1 and HBS2 and some sample

In Picture 5.6 above, second step or primary inner, tried some positive control and sample was conducted by conventional PCR with temperature of annealing 550C-650C, result of elektroforesis was not got by product of amplification single ribbon for temperature under 650C, but there are some ribbon giving positive result counterfeit alternatively was done by method of PCR with PCR nested for first phase use primary of P7 and P8 as primary outer hereinafter HBS1 and HBS2 as PCR inner with temperature of annealing 650C in each its phase. At first phase it was not found specific ribbon for positive control, but it was found

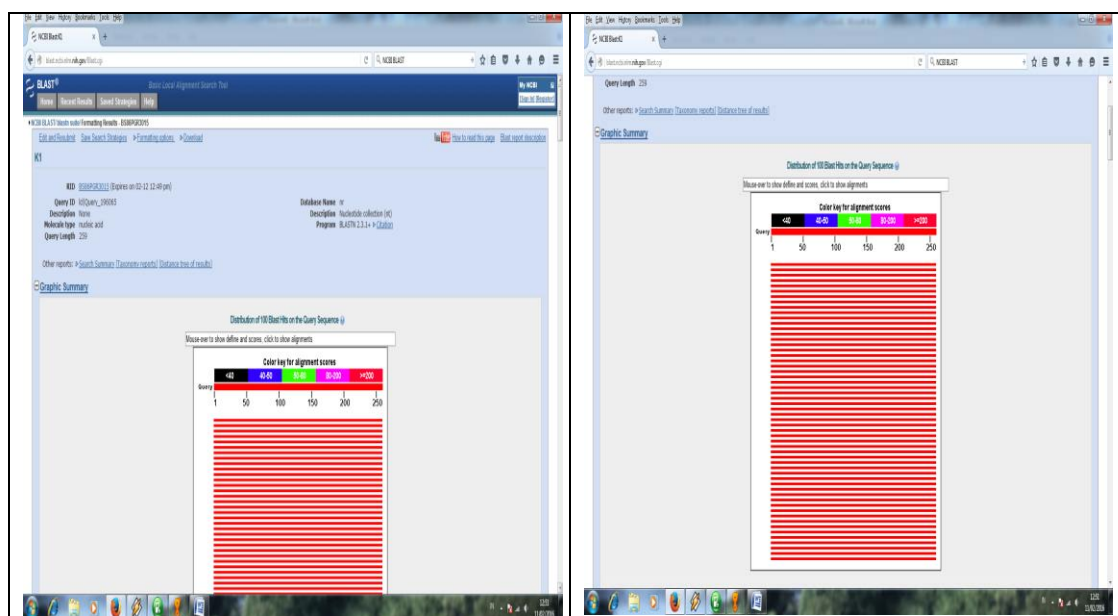
in second phase of single ribbon with product 100 bp for positive control.

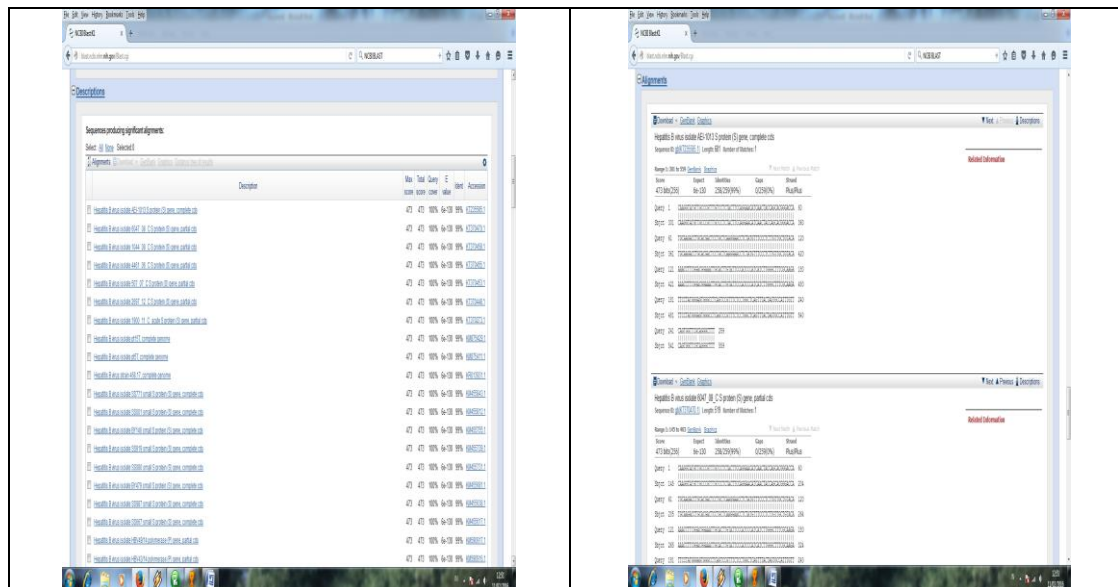
To ensure that positive control which is true virus of Hepatitis B, this DNA specimen was hereinafter sent to Macrogene, Republic Korea for the inspection of sequencing. Result of positive control sequencing can be seen in picture 5,7 hereunder.



**Picture 5.7:** Result of positive control sequencing

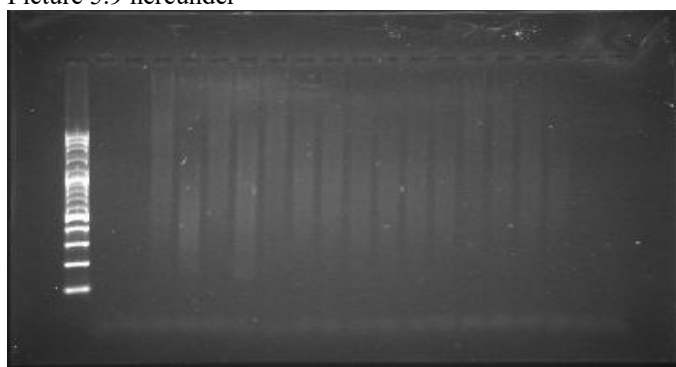
In Picture 5.7 above, it can be seen the result of sequencing of positive control. Result of this sequencing is processed by using program of BLAST, as seen at Picture 5.8 hereunder.





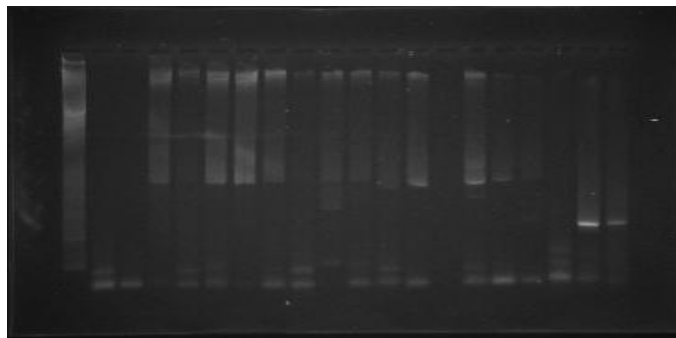
**Picture 5.8:** Result of analysis of sequencing positive control

In Picture 5.8 above, it can be expressed that positive control which used in this research is Virus of Hepatitis B (VHB). After getting result of Primary optimasi with temperature 650C, then inspection was done at all sample, with temperature of annealing 650C for all research phase. Result of conventional PCR at first phase can be seen in Picture 5.9 hereunder



**Picture 5.9:** Result of PCR sample (18-31) primary dg P7 + P8 with temperature of Annealing 650C

In Picture 5.9 above can be seen the result of positive control and sample and also negative control was not be obtained by ribbon at its result. Then, the second PCR phase was conducted by using primary of HBS1 and HBS2. Result of this second phase elektroforesis can be seen in Picture 5.10 hereunder.



**Picture 5.10:** Result of PCR sample ( 18-31) Primary dg of HBS1+HBS2 with temperature

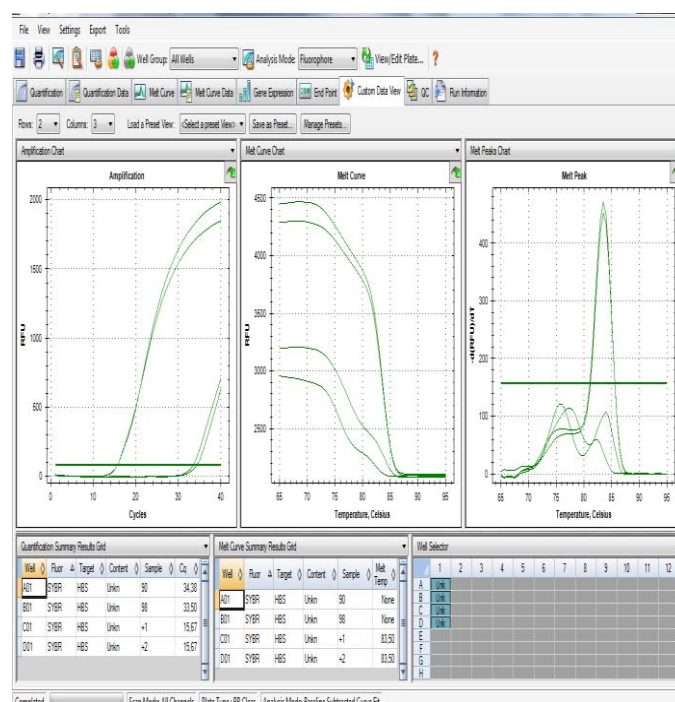
Annealing 650C ( L100Bp, left side control - 2 well and right side control + 2 well

In Picture 5.10 above can be seen that result of conventional PCR second phase was not found in DNA VHB at checked sample. Result of all sample can be seen in enclosure 4.

### 5.3 Occult Hepatitis B in donor blood with negative HBSAG and Anti positive HBS

After all sample was done by inspection of conventional PCR, in DNA VHB elektroforesis, which there are smooth ribbon which its position close to positive control line ( 100bp), then conducted inspection of RT PCR, to determine whether the flimsy ribbon represent DNA VHB or not.

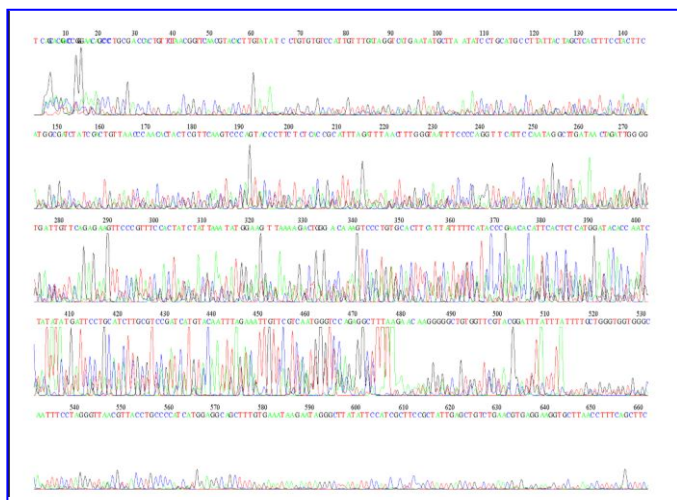
Result of inspection of RT PCR can be seen in picture hereunder.



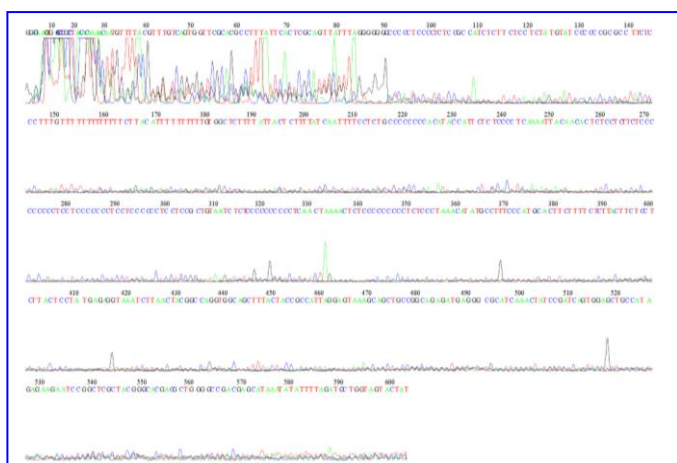
**Picture 5.11:** Result of inspection RT PCR in DNA VHB

Inspection with RT PCR can be seen in Picture 5.11 above, (Result of RT PCR entirely can be seen at Enclosure). The inspection of RT PCR was found by the sample coming near its result with positive control. But to ascertain what is result of this RT PCR in DNA VHB, it was done by sequencing to no sample 90 and 98 above

Result of no sample sequencing 90 and none 98 can be seen in Picture 5.13 and Picture 5.14 hereunder.

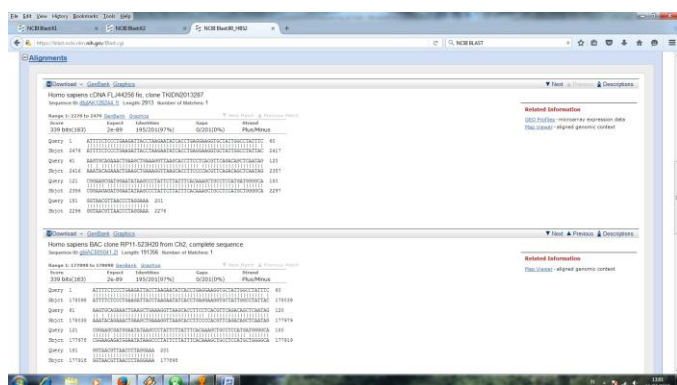


**Picture 5.13:** Result of no sampel sekuensing. 90



**Picture 5.14:** Result of no sample sequencing 98

Result of inspection of DNA no sample sequencing 98 and 90 processed to use program of BLAST, can be seen in picture 5.15 hereunder.



## 6. Solution

### 6.1 DNA VHB in Blood Donor with Negative HBSAG and positive Anti hbs

Research Sample was counted 375 sample which have been conducted inspection of HBSAG and expressed negativity obtained from 2 UTDRS. Hereinafter, the sample was done inspection of anti HBs. Result of inspection anti HBS was positive found in 238 sample, later; then the specimen was conducted by inspection of insulation of DNA

Result of insulation DNA to 238 sample, coming from UTD RSUD Prof.Dr MA Hanafiah SM Batusangkar counted 128 sample and from UTD RSUD Sawahlunto counted 110 sample

To determine efficacy of insulation at all sample which have been done insulation of DNA VHB, inspection of concentration DNA by using drops none 2000 spectrophotometer. Result of concentration of DNA obtained vary, from 0,5 ng /  $\mu$  L - 34,6 ng /  $\mu$  L.

In this research, result of insulation DNA VHB also can be seen by using gel of electrophoresis 1,2% in buffer 0,5x TBE. Result of insulation DNA can be seen in picture 5.2

Pursuant to result of inspection of drops none and inspection of electrophoresis, evaluated from bibliography, inspection of insulation expressed to succeed, so that sample can be continued for inspection hereinafter.

Conducting inspection in laboratory have top notch if accuracy and its good. The Control quality of inspection of PCR was done with inspection of negative and positive control.

For positive control of DNA VHB, used control of real correct sample of positive correctness have DNA VHB, expressed with inspection of positive conventional PCR, positive RT PCR and result of sequencing. For negative control, used sterile acuades.

Inspection of PCR by using primary of P7 and P8 was done with temperature of annealing 650C, this matter disagree with guide of factory and also from research of other research which use same primary, they use temperature 550C. Of course stipulating of high temperature represent stipulating of new temperature, because with temperature 650C it got single ribbon at its result.

Pursuant to stipulating of the temperature above, as according to bibliography, that inspection of PCR represent an art, depended appliance of PCR used, and also ability of human resource, so that make a good living accuracy of usage of correct temperature to a[n inspection.

Result of inspection of donor blood sample use conventional PCR, was not found in DNA VHB

Result of this inspection oppose against research done by Badrawy in Egypt 2013, where Badrawy find 2 from 405 sample, with positive DNA VHB, but Badrawy express that 2 of the sample which are positive have rate of anti HBs which is low. Result of this research also oppose against



research of Theja year 2010, where Theja find 25 sample which are positive DNA VHB from 309 sample with negative HBSAG, but positive anti HBs titer lower.

Pursuant to bibliography, anti HBs formed in six infection of VHB. With formed of antiHBs, depended formed antibody titer, hence there are some possibility, first among others is recovering perfection, both of DNA VHB there is still but low in number or which is recognized with VHB occult. And hereinafter superinfeksi with VH mutant. Gain strength formed anti HBs titer, hence is big possibility to recover perfection

Pursuant to bibliography, checked anti HBs use strip tets have strong anti HBs titer. Result of this research as according to bibliography above, that anti HBs with strong titer can express someone recover perfection, proven mentioned in this research, it was not found by DNA VHB, so that need inspection of anti HBs with strong titer for the skringing of donor blood if [at] inspection of negative HBSAG

Pursuant to bibliography, checked anti HBs use strip test have strong anti HBs titer. Result of this research as according to bibliography above, that anti HBs with strong titer can express someone recover perfection, proven mentioned at this research, none found DNA VHB, so that need inspection of anti HBs with strong titer for the skringing of donor blood if inspection of negative HBSAG

Result of this research oppose against research of Mahtab in the year Bangladesh, where found DNA VHB with RT PCR at 8 from 20 cirrhosis patient of hepatis, 2 from 10 people with make-up of rate of Alanin trasferase ( ALT

Result of this research nor chime in with research of Brechot in 1985 in India, where Brechot find 52 from 88 patient of B chronic hepatitis with OBI.

Mahtab and Brechot in its research use sample patient of Hepatitis B chronic, some among others there are cirrhosis complication of hepatitis. Of course intake of sampel differ from research, where researcher sample is sampel from one who is categorized healthy to be conducted by blood donor.

Result of research got that there is no DNA VHB and also occult Hepatitis B in donor blood with negative HBSAG and Anti positive HBS.

However this research have weakness also, that compared to research of others, researcher use primary which commonly use to detect VHB with C genotype and of B, what epidemiology spread over in Indonesia, while research of others by using primary for the VHB OF A genotype, B and of D.

The election of correct primary as according to VHB genotype very require to be paid attention as according to epidemiology spreading of VHB in world, although it was not closed possibility of its existence of E genotype, F, G, H, and I which during the time reside in America, China and Europe will be found in Indonesia

## 7. Conclusion and Suggestion

### 7.1 Conclusion

Result of research which have been done can be taken by the following conclusion 1. There are DNA VHB in donor blood with negative HBSAG and anti positive HBS 2. there are no Hepatitis B occult in donor blood with negative HBSAG and anti positive HBS

### 7.2 Suggestion

- 1) Require to be enhanced inspection of anti HBs for the skringing of donor blood with inspection of negative HBSAG in UTD RS to prevent infection of VHB
- 2) Require to be thought of to use PCR for the skringing of donor blood so that infection of contagious disease through blood can be prevented.

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