

SUMMARY
PROTOCOL FOR
PRODUCTION AND TESTING OF
HEPATITIS B VACCINES MADE
BY RECOMBINANT DNA
TECHNIQUES

Based on
Requirements for Biological
Substances No. 45(Requirements for Hepatitis B Vaccines
Made by Recombinant DNA Techniques,WHO Technical
Report Series, No. 786, 1989,
Annex 2)

KOREA

CERTIFICATE

I certify that Summary protocol for production and testing of Euvax B is based on the analysis data written in Korean, and all data were translated into English correctly.

Confirmed by



Hyung ryul Jang
Responsible QA manager
LG Chem.

03 Apr.2019

<i>Identification of final lot;</i>	UVA18013
<i>Name and address of manufacturer;</i>	LG Chem. /Iksan Plant #601, Yongje-dong, Iksan-si, Jeonbuk-do, 570-350 Korea
<i>International name and proprietary name of vaccine;</i>	Vaccinum hepatitis B recombinatum, Euvax-B
<i>Date of manufacture of final lot;</i>	21 Dec.2018
<i>Date of filling containers;</i>	27 Dec.2018
<i>Number and nature of containers (ampoules or vials);</i>	219389 Vials
<i>Date of last potency test;</i>	15 Jan.2019
<i>Number of doses in each container;</i>	1 dose / Vial
<i>Volume of single dose;</i>	0.5 mL
<i>Expiry date;</i>	20 Dec.2021

3. Validation and control of manufacturing procedures

3.1 Strategy for cloning and expressing the gene

Details of:

(a) potential retrovirus-like particles in and genetic markers of the host cell;

Method : Reverse transcriptase assay
Results : No RT activity

(b) construction, genetics, and structure of the expression vector;

Data #1

(c) origin and identity of the gene that was cloned

Origin : Genomic DNA of HBV from blood of infected patient
Identification : DNA sequencing

Description, in detail, of the measures taken to promote and control the expression of the cloned gene in the host cells;

ADH2/GAP promotor
Ethyl alcohol level control

Information on the stability of the expression system during storage and cell propagation to the production level;

Summary of 7 batches
Optical Density of cell at the end of culture : 69.7 ~ 84.7
Expression level (by EIA(AUSZYME)) :
Plasmid retention level test :
Passed

3.2 Biochemical characterization of recombinant vector

Nucleotide sequence of the surface-antigen gene insert ;

Data #2

Restriction-endonuclease mapping of the recombinant vector, where relevant;

Data #3

3.3 Purification procedures

Methods used to purify the HBsAg;

Results; | Data #4

Origins and characteristics of antibodies,

if use; | Unnecessary

3.4 Characterization of gene products (HBsAg)

3.4.1 Particle characterization

Morphological characteristics of the particles

*Results of examination by electron
microscopy*

Average 20nm particles, homogeneous

Degree of aggregation

Method;

Results;

CsCl₂ density gradient ultracentrifugation

50 ~ 100 polypeptides per particle

Density : 1.2 (g/mL)

Quantity of protein

Method;

Results;

Lowry method

463 ug/mL UVB18022

475 ug/mL UVB18023

Quantity of lipid

Method;

Results;

Sulfur-phospho-Vanillin Assay

57 ug/100ug UVB18022

66 ug/100ug UVB18023

Quantity of nucleic acid

Method;

Results;

Immuno-ligand assay (Threshold system)

0 pg/20 ug UVB18022

1 pg/20 ug UVB18023

Quantity of carbohydrate

Method; Anthron -Sulfuric acid Assay
Results; 3 ug/100ug UVB18022
4 ug/100ug UVB18023

3.4.2 Determination of protein content (HBs Ag)

Method; ECLIA
Results; Not lower than standard

3.4.3 Protein characterization

Ultraviolet absorption spectrum; Data #5
Protein composition
Method; Image scanning after SDS-PAGE
Results; Purity: Not less than 95%

Identity of the protein by partial N-terminal and C-terminal analysis

Method; Edman degradation
Results; N terminal : MESTTSGFLG
C terminal : I

3.4.4 Antibodies induced by the vaccine in human beings

Titres
Characteristics (Clinical data); Data #6

3.4.5 Consistency of yield

Between runs
During individual runs; Summary of 10 batches(bulk production)
(Average \pm standard deviation)
Yield deviation less than 25%
1) Extraction: 91gram \pm 6 gram
2) pH Cut: 102 \pm 6 %
3) Silica desorption: 29 \pm 3 %
4) Column 1: 56 \pm 5 %

5) Column 2: 75±8 %

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks

<i>Date of establishment of cell banks;</i>		23 Nov.2015 UVB18022 23 Nov.2015 UVB18023
<i>Quantity of cells stored;</i>		162 ea UVB18022 162 ea UVB18023
<i>Passage level of the MWCB;</i>		3 passages
<i>Storage conditions;</i>		-120 °C

4.2 Characteristics of cell seed lot

Purity and homogeneity of the cell seed lot		
<i>Method;</i>		DNA sequencing
<i>Results;</i>		Restriction enzyme mapping Phenotype test
Genetic characteristics of the cell seed lot		Plasmid retention
<i>Method;</i>		Non-contamination test
<i>Results;</i>		Expression level test All Passed
Purity of recombinant DNA vector		
<i>Method;</i>		Optical density check of purified plasmid
<i>Results;</i>		DNA Passed
Genetic characteristics of recombinant DNA vector		
<i>Method;</i>		Auxotrophic marker test
<i>Results;</i>		Passed
<i>Nucleotide sequence of HBsAg gene insert;</i>		Data #2
<i>Peptide map of gene product (HBsAg);</i>		Data #7
<i>Terminal amino acid sequence of gene produ</i>		Same as item 3.4.3

ct;

|

4.3 Phenotypic indicators of purity and genetic consistency of recombinant cultures

Tests on cells after recovery
from preserved state

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Method;

Auxotrophic marker test

Results;

|

Passed

Identity of cell seed

|

Method;

DNA sequencing

Results;

|

Passed

4.4 Sterility tests

Results;

|

Passed

4.5 Additional tests for MWCB of mammalian cells

|

Unnecessary

5. Production precautions

5.1 Production cell cultures;

|

Cell No.:

|

WHBV-11-63-082 UVB18022

|

WHBV-11-63-083 UVB18023

|

|

Room B-105

5.2 Culture conditions for production cell cultures

Cell doubling time;

4.72 hours

Number of subcultures;

3

Duration of permitted subculture

subculture 1 : 54 ±4 hours

subculture 2 : 16 ±4 hours

mainculture : 48 ±6 hours

Incubation temperature;

subculture 1 & 2 : 30 °C

Non-contamination test	main culture : 27°C
<i>Media;</i>	Microscopic examination
<i>Results;</i>	passed

5.3 Cell culture medium

	Unnecessary
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6. Single harvests

6.1 Non-contamination test performed at the end of the culture

<i>Media;</i>		Selection media
<i>Results;</i>		Passed

6.2 Consistency of yield of HBsAg

<i>Method;</i>		ECLIA
<i>Results;</i>		Deviation less than 25%

6.3 Plasmid retention

<i>Method;</i>		Auxotrophic marker test
<i>Results (proportion of cells still possessing the plasmid at the end of the culture);</i>		100%

7. Purification

<i>Methods;</i>		Data #4
<i>Results;</i>		Passed

7.1 Protein and other components of the vaccine

Protein content

<i>Methods;</i>	Lowry method
<i>Specification;</i>	400~600 μg/mL

Results; 463 ug/mL UVB18022
475 ug/mL UVB18023

Lipid content

Methods; Sulfur-phospho-Vanillin Assay
Specification; Not more than 100 μ g/ 100 μ g protein
Results; 57 ug/100ug UVB18022
66 ug/100ug UVB18023

Carbohydrate content (as compared with total proteins)

Methods; Anthron -Sulfuric acid Assay
Specification; Not more than 10 μ g / 100 μ g protein
Results; 3 ug/100ug UVB18022
4 ug/100ug UVB18023

Residual amounts of animal serum

Methods; Unnecessary
Results;

7.2 Tests for agents used during purification or other phases of manufacture

Methods; | Unnecessary
Results;

7.3 Determination of HBsAg content

Methods; | ECLIA
Specification; | Not lower than standard
Results; | Not lower than standard

7.4 Test for antigenic identity

Methods; | SDS-PAGE Mw : about 24KD
Specification; | Molecular weight of major band is about 2
4KD

Results; Passed

7.5 Test for sterility of purified surface antigen

Methods; | Membrane filtration method

Specification; | Sterile

Results; Passed

7.6 Test for inactivating agents

Methods; | Unnecessary

Results;

8. Final bulk before addition of adjuvant (aqueous bulk)

Volume of purified bulk | Not performed

Number of batches pooled | Not performed

Nature and volume of diluent added

8.1 Test for sterility

Methods; | Not performed

Results;

8.2 Test for HBsAg

Quantity of HBsAg | Not performed

Methods; |

Results;

Ratio of HBsAg to total protein | Not performed

Methods; |

Results;

8.3 Test for DNA

Not performed

Methods; |

Results;

9. Final bulk (UVN18005)

9.1 Addition of adjuvant

Volume of bulk;

9364.670 G UVB18022

9777.720 G UVB18023

Nature and volume of adjuvant added

Aluminum hydroxide gel

24741 G

and final concentration;

to 0.15%

Nature and volume of preservative added

Thimerosal

44.9 G

and final concentration;

0.01%

Composition of final bulk

mixing of all ingredients) and identification number;

Purified HBV :

UVB18022

UVB18023

Aluminium hydroxide gel :

Y170630007

Y180320014

Thimerosal :

Y180315012

Potassium phosphate monobasic :

Y180322020

Sodium phosphate dibasic :

Y180711009

Sodium chloride :

Y181120001

9.2 Tests on final bulk

9.2.1 Tests for sterility		
<i>Methods;</i>		Membrane filtration method
<i>Specification;</i>		Sterile
<i>Results;</i>		Passed
9.2.2 Tests for preservatives		
<i>Methods;</i>		Chemical assay
<i>Specification;</i>		Not more than 0.0115w/v %
<i>Results;</i>		0.0103 w/v%
9.2.3 Assay for adjuvant		
<i>Methods;</i>		Chemical assay
<i>Specification;</i>		Not more than 1.25mg/mL
<i>Results;</i>		0.52 mg/mL

10. Filling and containers

<i>Date of filling;</i>		27 Dec.2018
<i>Quantity of containers;</i>		219389 Vials
<i>Volume of vaccine per container;</i>		0.5 mL
<i>Control for defective containers;</i>		Seidenader-PI-40

11. Control of final lot

11.1 Appearance		
<i>Methods;</i>		Naked eye
<i>Specification;</i>		Well-dispersed suspension
<i>Results;</i>		Passed
11.2 Sterility		

<i>Methods;</i>	Membrane filtration method
<i>Specification;</i>	Sterile
<i>Results;</i>	Passed
11.3 Test for pyrogenic substances	
<i>Methods;</i>	Rabbit test
<i>Specification;</i>	Meets the requirement
<i>Results;</i>	Passed
11.4 Test for pH	
<i>Methods;</i>	pH meter
<i>Specification;</i>	5.4 ~ 7.4
<i>Results;</i>	6.5
11.5 Innocuity tests	
In mice	
<i>No. and weight of animals;</i>	5 animals 19.6 → 28.6 18.8 → 27.9 20.6 → 29.8 18.6 → 25.6 20.0 → 30.6
<i>Quantity injected;</i>	1.0 mL
<i>Observation period;</i>	1 week
<i>Specification;</i>	Meets the requirement
<i>Result</i>	Passed
In guinea-pigs	
<i>No. and weight of animals;</i>	2 animals 301.7 → 341.7 309.4 → 345.4
<i>Quantity injected;</i>	5 mL
<i>Observation period;</i>	1 week
<i>Specification;</i>	Meets the requirement
<i>Result</i>	Passed
11.6 Assay for adjuvant	
<i>Methods;</i>	Chemical assay
<i>Specification;</i>	Not more than 1.25mg/mL

<i>Results;</i>	0.51 mg/mL
11.7 Assay for protein content	
<i>Methods;</i>	Lowry method
<i>Specification;</i>	Not more than 40 μ g/mL
<i>Results;</i>	23 ug/mL
11.8 Test for preservative	
<i>Methods;</i>	Chemical assay
<i>Specification;</i>	Not more than 0.0115w/v %
<i>Results;</i>	0.0101 w/v%
11.9 Potency and identity tests	
Identification of vaccine as HBsAg	
<i>Methods;</i>	ECLIA
<i>Results;</i>	Identified
Antigen content	
<i>Methods;</i>	ECLIA
<i>Specification;</i>	Relative potency should be not less than 0.5
<i>Results;</i>	0.9

CERTIFICATE

I, the undersigned, certify that lot no. UVA18013 of the vaccine satisfies Part A of the *Requirements for Hepatitis B Vaccines made by Recombinant DNA Techniques* published by WHO.

Confirmed by



Hyung ryul Jang
Responsible QA manager
LG Chem.

03 Apr.2019