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

Identification of final lot;	UVA18012
Name and address of manufacturer;	LG Chem. /Iksan Plant #601, Yongje-dong, Iksan-si, Jeonbuk-do, 570-350 Korea
International name and proprietary name of vaccine;	Vaccinum hepatitidis B recombinatum, Euvax-B
Date of manufacture of final lot;	20 Dec.2018
Date of filling containers;	26 Dec.2018
Number and nature of containers (ampoules or vials);	219397 Vials
Date of last potency test;	15 Jan.2019
Number of doses in each container;	1 dose / Vial
Volume of single dose;	0.5 mL
Expiry date;	19 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 retension 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;*	$CsCl_2$ 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

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

5) Column 2: 75±8 %

4. Manufacturer's working cell bank (MWCB)

4.1 Origin of cell banks

Date of establishment of cell banks;	23 Nov.2015 UVB18022 23 Nov.2015 UVB18023
Quantity of cells stored;	162 ea UVB18022 162 ea UVB18023
Passage level of the MWCB;	3 passages
Storage conditions;	-120℃

4.2 Characteristics of cell seed lot

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

	main culture ： 27℃

Non-contamination test

Media;	Microscopic examination
Results;	passed

5.3 Cell culture medium

	Unnecessary

6. Single harvests

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

Media;	Selection media
Results;	Passed

6.2 Consistency of yield of HBsAg

Method;	ECLIA
Results;	Deviation less than 25%

6.3 Plasmid retention

Method;	Auxotropic marker test
Results (proportion of cells still possessing the plasmid at the end of the culture);	100%

7. Purification

Methods;	Data #4
Results;	Passed

7.1 Protein and other components of the vaccine

Protein content

Methods;	Lowry method
Specification;	400~600μg/mL

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

Lipid content

Methods;	Sulfur-phospho-Vanillin Assay
Specification;	Not more than 100μg/ 100μgprotein
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 10μg / 100μg 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 24KD

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%
Nautre and volume of preservative added	Thimerosal 44.9 G
and final concentration;	0.01%
Composition of final bulk *mixing of all ingredients) and identification n umber;*	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	
Methods;	Membrane filtration method
Specification;	Sterile
Results;	Passed
9.2.2 Tests for preservatives	
Methods;	Chemical assay
Specification;	Not more than 0.0115w/v %
Results;	0.0103 w/v%
9.2.3 Assay for adjuvant	
Methods;	Chemical assay
Specification;	Not more than 1.25mg/mL
Results;	0.52 mg/mL

10. Filling and containers

Date of filling;	26 Dec.2018
Quantity of containers;	219397 Vials
Volume of vaccine per container;	0.5 mL
Control for defective containers;	Seidenader-PI-40

11.Control of final lot

11.1 Appearance	
Methods;	Naked eye
Specification;	Well-dispersed suspension
Results;	Passed
11.2 Sterility	

Methods;	Membrane filtration method
Specification;	Sterile
Results;	Passed
11.3 Test for pyrogenic substances	
Methods;	Rabbit test
Specification;	Meets the requirement
Results;	Passed
11.4 Test for pH	
Methods;	pH meter
Specification;	5.4 ~ 7.4
Results;	6.5
11.5 Innocuity tests	
In mice	
No. and weight of animals;	5 animals
	22.4 → 32.4
	19.0 → 28.0
	19.6 → 30.4
	18.4 → 28.1
	20.9 → 30.8
Quantity injected;	1.0 mL
Observation period;	1 week
Specification;	Meets the requirement
Result	Passed
In guinea-pigs	
No. and weight of animals;	2 animals
	279.9 → 317.4
	375.3 → 309.8
Quantity injected;	5 mL
Observation period;	1 week
Specification;	Meets the requirement
Result	Passed
11.6 Assay for adjuvant	
Methods;	Chemical assay
Specification;	Not more than 1.25mg/mL

Results;	0.51 mg/mL
11.7 Assay for protein content	
Methods;	Lowry method
Specification;	Not more than 40μg/mL
Results;	23 ug/mL
11.8 Test for preservative	
Methods;	Chemical assay
Specification;	Not more than 0.0115w/v %
Results;	0.0105 w/v%

11.9 Potency and identity tests

Identification of vaccine as HBsAg

Methods;	ECLIA
Results;	Identified

Antigen content

Methods;	ECLIA
Specification;	Relative potency should be not less than 0.5
Results;	0.8

CERTIFICATE

I, the undersigned, certify that lot no. UVA18012 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