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Producer of immunological veterinary medicinal products
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ANALYTICAL CERTIFICATE

ROTAVAK vakcina A.U.V. **Batch: 85 06 18 01**

Expiry: 05.06.2020

TESTING	METHOD	STANDARD	RESULT
Biological parameters			
Sterility	528-III/07	Absence of growth of micro-organisms.	Absence of growth of micro-organisms.
Potency In rabbits / Serology	528-III/09	Vaccination of rabbits by 2 ml of the vaccinal dose stimulates an increase of VN antibody titre: BRV min. 6.2 - max. 9.0 log_2 HI antibody titre: BCV min. 5.3 - max. 8.0 log_2 ELISA antib. titre: F5 E.coli min. 43.5% - max. 95.2%*	Vaccination of rabbits stimulated an increase of antibodies: BRV: 7,1 log2 BCV: 7 log2 F5 E. coli: 91 % *
Identity In rabbits / Serology	528-III/15	Specific post-vaccination antibodies against vaccine antigens.	Specific post-vaccination antibodies against vaccine
Extraneous viruses In target animals	528-III/14	No increase of specific antibodies against BHV-1, BLV and BVDV.	No increase of specific antibodies against BHV-1, BLV and BVDV.
Physicochemical parameters			
Appearance	528-III/05	Emulsion of white colour, formation of easily shakeable sediment is allowed.	Emulsion of white colour, formation of easily shakeable sediment is allowed.
Extractable volume	528-III/06	15 ml	15 ml
Viscosity	528-III/16	15 – 30 mPa.s	20,21 mPa.s
pH value	528-III/10	pH 6.0 – 7.5	pH 6,94
Content of formaldehyde	528-III/11	maximum 0.5 g/l	0,39 g/l
Content of thiomersal	528-III/12	0.08 – 0.12 g/l	0,10 g/l
Stability of emulsion	528-III/13	Emulsion is stable and type water-in-oil-in-water.	Emulsion is stable and type water-in-oil-in-water.
Content of adjuvant	528-III/18	54 ± 2.5 %	54,00 %

* expressed as % of inhibition

- CONFORMING -

I herewith certify that **ROTAVAK vakcina A.U.V. , Batch: 85 06 18 01** was manufactured and tested and complies with the quality requirements given in Marketing Authorization Dossier as they were approved by the competent authorities and that all measures have been taken to demonstrate compliance with Directive 2001/82/EC as amended by Directive 2009/9/EC.

Nitra, July 17, 2018

PHARMAGAL BIO s.r.o.
Murgašova 5
949 01 NITRA

MVDr. Denisa Svitačová
Quality Control Laboratory

ŽIADOSŤ O ÚRADNÉ UVOĽNENIE ŠARŽE IVL NA TRH EÚ

Request for OBPR – Procedure for a harmonised application of art. 81 for official batch release of IVMP in the EU

According to Art. 81 of Council Directive 2001/82/EC amended by Directive 2004/28/EC

Protokol výrobcu o šarži lieku

MANUFACTURERS BATCH PROTOCOL

1. Všeobecné informácie
MEMBER STATE SPECIFIC INFORMATION

Žiadateľ - Meno a adresa žiadateľa/držiteľa registračného rozhodnutia *Name and address of marketing authorisation holder*	Newcopharm Hungária Kft. Rákóczi u. 142-146 7100 Szekszárd
Výrobca (meno, adresa výrobcu) ak sa líši od držiteľa *Name and address of manufacture, if different*	Pharmagal Bio, s. r. o., Murgašova 5 949 01 Nitra
Obchodný názov imunologického veterinárneho lieku *Trade name:*	ROTAVAK vakcina A.U.V.
Medzinárodný názov / liekopisný / všeobecný názov *International non-proprietary Name / Ph. Eur. name / common name*	Inactivated vaccine Bovine rotavirus + bovine coronavirus + E.coli ATCvet. code: QI02AL01
Lieková forma *Pharmaceutical form of finish. prod.*	Suspension for injection
Skladovacia teplota *Storage temperature*	+2 - +8°C Do not freeze.
Registračné číslo a dátum vydania registračného rozhodnutia pre vakcínu/riedidlo *Marketing authorisation number for vaccine/diluent (Member State / EC) issued by*	3825/1/16 NÉBIH ÁTI (5 adag)

2. Súhrnné informácie o šarži hotového výrobku
SUMMARY INFORMATION ON THE FINAL BATCH OF FINISHED PRODUCT

Číslo šarže vakcíny *Manufacturer's batch number(s) appearing on package and other identification numbers associated with this batch (final bulk no, final lot no, packaging lot no)*	85 06 18 01 Final batch from Bulk - 85 06 18
Číslo šarže riedidla *Batch nr. of diluent (where appropr.e)*	Not appropriate
Druh obalu *Type of container:*	Glass vials (hydrolytic resistance Type I), rubber chlorobutyl stoppers and aluminium cap with central tear-off
Veľkosť šarže a počet obalov pre jednotlivé veľkosti balenia *Total number of containers in this batch*	7 500 pcs
Počet obalov uvoľňovaných na trh *Number of containers the release is applied for*	1 200 pcs
Počet dávok/objem *Nr. of doses /volume per container:*	5 vaccine doses / 15 ml
Dátum začiatku platnosti doby exspir. (deň miešania) *Date of start of period of validity (day of blending)*	6.6.2018
Dátum exspirácie *Date of expiry*	05/06/2020

3 PRODUCTION INFORMATION

Site of manufacture for each antigen (whenever more than one production site exists): Identical

3.1 COMPONENTS

3.a BRV COMPONENT

3.a.1. STARTING MATERIALS

3.a.1.1 Virus seed lots

Master seed material: BRV - TM91 MS-batch number: MVS BRV TM 91 02/06 Prepared: 15.8.2006
Date of last testing: 15.08.2006-03.11.2006
Working seed material: BRV - TM91 WS-batch number: WVS BRV TM 91 02/06 Prepared: 22.8.2006
Date of last testing: 22.08.2006-03.11.2006

3.a.1.2. Substrates

3.a.1.2 Cell line MA 104

Master seed material: MA 104 MCS-batch number: MCS MA104 02/05 Prepared: 16.3.2005
Date of last testing: 15.08.2006-03.11.2006
Working seed material: MA 104 WCS-batch number: WCS MA104 02/05 Prepared: 23.3.2005
Date of last testing: 23.03.2005; 03.11.2006

3.a.2 INTERMEDIATE STAGES OF PRODUCTION

3.a.2.1.Production of antigen BRV

Batch of antigen Production step	Start	End	Material	Quantity
21 05 18 Seed	21.5.2018	23.5.2018	MA 104 TM - 91	326 Roux 2 ml 1:10
Harvest	23.5.2018		Infectious fluids BRV TM-91	40 763 ml
23 05 18 Inactivation	23.5.2018	28.5.2018	Infectious fluids BRV TM-91 10 % formladehyde	40 750 ml 408 ml

3.b BCV COMPONENT

3.b.1. STARTING MATERIALS

3.b.1.1 Virus seed lots

Master seed material: BCV - C197 MS-batch number: MVS BCV C-197 02/06 Prepared: 23.8.2006
Date of last testing: 23.08.2006-03.11.2006
Working seed mat.: BCV - C197 WS-batch number: WVS BCV C-197 02/06 Prepared: 30.8.2006
Date of last testing: 30.08.2006-03.11.2006

3.b.1.2. Substrates

3.b.1.2 Cell line EBT

Master seed material: EBT MCS-batch number: MCS EBT 02/04 Prepared: 14.4.2004
Date of last testing: 23.08.2006-03.11.2006
Working seed material: EBT WCS-batch number: WCS EBT 02/04 Prepared: 22.4.2004
Date of last testing: 23.03.2005; 03.11.2006

3.b.2 INTERMEDIATE STAGES OF PRODUCTION

3.b.2.1.Production of antigen BCV

Batch of antigen Production step	Start	End	Material	Quantity
18 05 18 Seed	18.5.2018	22.5.2018	EBT C-197	240 Roux 5 ml 1:100
Harvest	22.5.2018		Infectious fluids BCV C-197	27 795 ml
23 05 18 Inactivation	23.5.2018	28.5.2018	Infectious fluids BCV C-197 10 % formladehyde	27 790 ml 278 ml

3.c E. coli COMPONENT

3.c.1. STARTING MATERIALS

3.c.1.1 Bacterial seed lots

Master seed material: E. coli Ec17 MS-batch number: MBS Ec17 01/00 Prepared: 8.2.2000
Date of last testing: 08.02.2000, 18.05.2012
Working seed material: E. coli Ec17 WS-batch number: WBS Ec17 01/00 Prepared: 23.2.2000
Date of last testing: 08.02.2000, 18.05.2012

3.c.2 INTERMEDIATE STAGES OF PRODUCTION

3.c.2.1.Production of E.coli Ec17

Batch of antigen Production step	Start	End	Material	Quantity
Batch - **10 05 18** Growth onto solid medium	9.5.2018	10.5.2018	MINCA agar E. coli Ec17	3 plates 1 vials
Growth in liquid medium	10.5.2018	10.5.2018	MINCA broth	70 000 ml
Inactivation	10.5.2018	11.5.2018	10 % formaldehyde	3 682 ml

3.c.2.2.Concentration of inactivated antigens E.coli Ec17

Final batch of antigen	Production of antigens	Date of concentration	Material	Quantity
23 05 18	**10 05 18**	23.5.2018	Inactivated bacterial broth	10 100 ml

CREATION OF THE FINISHED PRODUCT

3.2.1 BLENDING AND VACCINE COMPOSITION

3.2.1.1. Adjusting antigens to target concentration

Date of adjusting: 6.6.2018

Antigen	Batch of antigen	Volume of antigen	Volume of PBS	Final volume adj. antigen
Inactivated BRV	23 05 18	30 800 ml	0 ml	30 800 ml
Inactivated BCV	23 05 18	28 050 ml	2 750 ml	30 800 ml
Inactivated E.coli F5	23 05 18	10 000 ml	20 800 ml	30 800 ml

3.2.1.2. Blending of the final bulk

Date of blending: 6.6.2018

Total volume: 200 000 ml

3.2.1.3. Composition of final bulk:

Batch number of the final bulk: 85 06 18

Component	Batch no.	Target Concentration.	Final Concentration.
Antigen			
Adjusted BRV	23 05 18	15,3% ± 0.33 %	15,3%
Adjusted BCV	23 05 18	15,3% ± 0.33 %	15,3%
Adjusted E.coli F5	23 05 18	15,3% ± 0.33 %	15,3%
Excipients			
5% thiomersal	49/18	0,2%	0,2%
Adjuvant			
Montanide ISA 206 VG	171218018147	54% ± 1 %	53,9%

3.3. FILLING

Batch number of final bulk used for fill: 85 06 18

Final batch No.	Filling date	Quatity of filled containeirs	Volume filled
85 06 18 01	25.6.2018	7 500 pcs	16,5 ml

3.4. IN PROCESS CONTROLS

3.4.1 In process controls on BRV TM-91

Titration of BRV TM-91 virus (Code 528-II/2.1)

Start of testing:	25.5.2018				
End of testing:	30.5.2018				
Limit of acceptance:	min $10^{5,40}$ $FAID_{50}$	max	$10^{7,20}$ $FAID_{50}$		
Results:	**$10^{6,00}$**				

Conclusion: Complies.

Sterility testing of live BRV antigens (Code 528-II/2.3)

Start of testing:	25.5.2018
End of testing:	8.6.2018

Limit of acceptance: Absence of growth of micro-organisms.

Results: **No growth.**

Conclusion: Complies.

Control of inactivation of BRV TM-91 virus (Code 528-II/2.4)

Date of start of examination:	1.6.2018
Date of end of examination:	21.6.2018

Limits of acceptance of results: In all passages any signs of specific cytopathic effect must absent. In the last passage no rotavirus-specific immunofluorescence must be observed.

Result: In all passages any signs of specific cytopathic effect were absent. In the last passage no rotavirus-specific immunofluorescence was observed.

Conclusion: Complies

Determination of the content of inactivated BRV after inactivation (Code 528-II/2.12)

Date of examination: 30.5.2018

Limits of acceptance of results: min. 0,9 O.D. - max. 1.9 O.D.

Result: **0,9**

Conclusion: Complies

Examination of sterility of inactivated BRV antigen (Code 528-II/15)

Date of start testing:	1.6.2018
Date of end testing:	15.6.2018

Limits of acceptance of results: Absence of growth of micro-organisms.

Results: **Absence of growth of micro-organisms.**

Conclusion: Complies

Determination of the content of inactivated BRV after blending (Code 528-II/2.16)

Date of examination: 6.6.2018

Limits of acceptance of results: 0.6 O.D.

Result: **0,6 O.D.**

Conclusion: Complies

3.4.2 In process controls on BCV C-197

Titration of BCV C-197 virus (Code 528-II/2.2)

Start of testing:	25.5.2018	
End of testing:	29.5.2018	

Limit of acceptance: min $10^{4,20}$ $TCID_{50}$ max $10^{6,70}$ $TCID_{50}$

Results: **$10^{5,00}$**

Conclusion: Complies.

Sterility testing of live BCV antigens (Code 528-II/2.3)

Start of testing:	25.5.2018	
End of testing:	8.6.2018	

Limit of acceptance: Absence of growth of micro-organisms.

Results: **No growth.**

Conclusion: Complies.

Control of inactivation of BCV C-197 virus (Code 528-II/2.5)

Date of start of examination:	1.6.2018	
Date of end of examination:	21.6.2018	

Limits of acceptance of results: In all passages any signs of specific cytopathic effect must be absent.

Result: In all passages no signs of specific cytopathic effect were observed.

Conclusion: Complies

Examination of sterility of inactivated BCV antigen (Code 528-II/15)

Date of start testing:	1.6.2018	
Date of end testing:	15.6.2018	

Limits of acceptance of results: Absence of growth of micro-organisms.

Results: **Absence of growth of micro-organisms.**

Conclusion: Complies

Determination of the content of inactivated BCV after inactivation (Code 528-II/2.13)

Date of examination:	30.5.2018	
Result:	7 **log_2**	

Limits of acceptance of results: Minimum 7 log2 - max. 8 log2.

Conclusion: Complies

Determination of the content of inactivated BCV after blending (Code 528-II/2.17)

Date of examination: 6.6.2018

Limits of acceptance of results: 5 log2 .

Result: **5 log_2**

Conclusion: Complies

3.4.3 In process controls of E.coli F5

Determination of optical density of the live suspension E.coli EC/17 (Code 528-II/2.14)

Date of examination 10.5.2018
Limits of acceptance of results: min. 2,5 - max. 4,5 O.D.
Results: **3,7 O.D.**
Conclusion: Complies.

Determination of CFU of E.coli Ec17 in the broth (Code 528-II/2.6.)

Start of testing: 10.5.2018
End of testing: 12.5.2018
Limits of acceptance of results: min. 10^8 CFU - max. 10^{10} CFU in ml
Results: **4,2 x 10^9**
Conclusion: Complies.

Determination of purity (Code 528-II/2.7)

Start of testing: 10.5.2018
End of testing: 11.5.2018
Limits of acceptance of results: After 24-hour-long incubation grayish mucous colonies of 3 – 5 mm in diameter without haemolysis must be observed on blood agar. The colonies cultivated on MacConkey agar must be of pink color, lactose-positive.
Results: **Complies.**

Verification of the presence of F5 adhesin (Code 528-II/2.8)

Date of testing: 10.5.2018
Limits of acceptance of results:
Glass slide agglutination must prove the presence of Escherichia coli adhesin F5 (K99).
Results: **Presence of E.coli adhesin F5 was proved.**
Conclusion: Complies.

Control of inactivation of bacterial strain - (Code 528-II/2.9.)

Start of testing: 14.5.2018
End of testing: 22.5.2018
Limits of acceptance of results: No growth of bacterial germs must be observed.
Result: **No growth of bacterial germs was observed.**
Conclusion: Complies.

Determination of the amount of F5 adhesin (Code 528-II/2.10)

Date of testing: 24.5.2018
Limits of acceptance of results: min. 0,9 – max. 1,1 O. D.
Result: **1,10 O.D.**
Conclusion: Conforms.

Determination of the amount of F5 adhesin after blending (Code 528-II/2.18)
Date of testing: 6.6.2018
Limits of acceptance of results: 0,8
Result: **0,8 O.D.**
Concluson: Conforms.

Determination of the amount of bacterial endotoxin (Code 528-II/2.11)
Date of testing: 6.6.2018
Limits of acceptance of results: Maximum 80 000 EU / ml.
Result: **Less than 80 000 EU/ml.**
Concluson: Conforms.

Sterility testing of the blend of antigens (Code 528-II/19)
Date of testing: 6.6.2018
Date of end testing: 20.6.2018
Limits of acceptance of results: Absence of growth of micro-organisms.
Results: **Absence of growth of micro-organisms.**
Concluson: Conforms.

Quick verification of volume filling (Code 528-II/20)
Date of testing: 25.6.2018
Limits of acceptance of results: Commercial size of 5 vaccine doses must contains - 16.5 ml
Result: **16.5 ml**
Concluson: Conforms.

Quick verification of vials closing (Code 528-II/21)
Date of testing: 25.6.2018
Limits of acceptance of results: Cap is located in the center of the vial neck, walls of the cap even the bottom edge of the cap fit tightly to the glass surface of the vial. The stopper below the cap must be positioned upright in the vial. Aluminium cap must not rotate.

Result:

Caps are in the center of vial necks, walls and bottom fit thigly to the glass surface of the vial. The stoppers are upright in the vials and cap don´t rotate.

Concluson: Conforms.

4 FINAL BATCH TESTING

4.1. Finished product in bulk

EXAMINATION OF BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES

Examination of potency - Code 528-III/17

Date of start of testing: 7.6.2018

Date of end of testing: 17.7.2018

Number of vaccinated rabbits: 10 pcs

Limits of acceptance: Administration of the vaccine to rabbits must evoke serological response, that complies with the limits related for the antigen.

Result:

ANTIGEN	SEROLOGICAL RESPONSE	LIMIT OF ACCEPTANCE
BRV (VNT)	**7,1 log_2**	min. 6,2 log_2 - max. 9 log_2
BCV (HIT)	**7,0 log_2**	min. 5,3 log_2 - max. 8 log_2
E.coli F5 % of inhibition (ELISA)	**91,0%**	min. 43,5 % - max. 95.2 % of inhibition

Conclusion: Complies

Examination of identity (Code 528-III/15)

Date of testing: 7.6.2018 - 17.7.2018

Examination is identical with Efficacy testing.

Limits of acceptance of results: Formation of specific antibodies to vaccine antigens.

Result: **Formation of specific antibodies to vaccine antigens.**

Conclusion: Complies

Extraneous viruses (Code 528-III/14)

Date of testing: 7.6.2018 - 13.7.2018

Limits of acceptance of results: In safety test the vaccine must not induce an increase of specific antibodies against BHV-1, BLV and BVDV.

Result:

ANTIGEN	OCCURENCE OF SPECIFIC ANTIBODIES AGAINST	
	BEFORE VACCINATION	AFTER VACCINATION
BHV-1	**Negative**	**Negative**
BLV	**Negative**	**Negative**
BVDV	**Negative**	**Negative**

Conclusion: Complies

EXAMINATION OF PHYSICOCHEMICAL PROPERTIES

pH value (Code 528-III/10)

Date of testing: 19.6.2018

Limits of acceptance of results: pH 6.0 – 7.5

Result: **pH 6,94**

Conclusion: Complies

Determination of the content of formaldehyde (Code 528-III/11)
Date of testing: 19.6.2018
Limits of acceptance of results: max. 0.5 g/l
Result: **0,39 g/l**
Conclusion: Complies

Determination of content of thiomersal (Code 528-III/12)
Date of testing: 19.6.2018
Limits of acceptance of results: 0.08 – 0.12 g/l
Result: **0,10 g/l**
Conclusion: Complies

Determination of content of adjuvant (Code 528-III/18)
Date of testing: 19.6.2018
Limits of acceptance of results: 54 ± 2.5 % v/v.
Result: **54,0%**
Conclusion: Complies

4.2. Batch of the finished product

EXAMINATION OF BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES

Examination of sterility (Code 528-III/7)
Date of start testing: 26.6.2018
Date of end testing: 10.7.2018
Limits of acceptance of results: Absence of growth of micro-organisms.
Results: **Absence of growth of micro-organisms.**
Conclusion: Complies

EXAMINATION OF PHYSICOCHEMICAL PROPERTIES

Appearance (Code 528-III/5)
Date of examination: 26.6.2018
Limits of acceptance of results: Emulsion of white colour, formation of easily shakable sediment is alowed.
Result: **Emulsion of white colour.**
Conclusion: Complies

Extractable volume (Code 528-III/6)
Date of examination: 25.6.2018
Limits of acceptance of results: For package size of 15 ml - 5 vaccine doses each of 3 ml.
Result: **5 vaccine doses each of 3 ml.**
Conclusion: Complies

Stability of emulsion, type of emusion (Code 528-III/13)
Date of testing: 19.6.2018
Limits of acceptance of results: Emulsion must be stable and type water-in-oil-in-water.
Results: **Emusion is stable. Type of emusion - w/o/w.**
Conclusion: Complies

Viscosity (Code 528-III/16)
Date of testing: 9.7.2018
Limits of acceptance of results: min. 15 mPa.s-max. 30 mPa.s
Result: **20,21 mPa.s**
Conclusion: Complies

5 CERTIFICATION BY THE MANUFACTURER

Certification by qualified person taking the overall responsibility for production and control of the product :

I herewith certify that ROTAVAK vakcina A.U.V. Batch N° 85 06 18 01 was manufactured and tested according to the procedures approved by the competent authorities and complies with the quality requirement and that all measures have been taken to demonstrate compliance with Directive 2001/82/EC as amended by Directive 2009/9/EC.

Name: MVDr. Denisa Svitačová
Function: Head of Quality Control Laboratory (Responsible for Batch Release)
Date: July 17, 2018

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949 01 NITRA

Signature: _____