MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

Federal State Budgetary Institution "National Research Centre for Epidemiology and Microbiology named after the honorary academician N.F.Gamaleya"
of the Ministry of Health of the Russian Federation
(N.F.Gamaleya NRCEM)

18 Gamaleya Street
Moscow, 123098
Russian Federation

Tel: 8 499-193-30-01
Fax:8 499-193-61-83

www.gamaleya.org
e-mail: info@gamaleya.org

May 3, 2021

To Antonio Barra Torres, President-Director
Brazilian Health Regulatory Agency (ANVISA)

Following the letter from União Química dated April 28, 2021, the Federal State Budgetary Institution N. F. Gamaleya National Research Center of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation ("Gamaleya Center") herewith would like to respond to ANVISA's allegations about the Sputnik V vaccine that have been made in public throughout the week of April 26th, specifically during the public meeting of ANVISA on April 26 and the press conference of ANVISA on April 29, which caused significant harm to reputation of Gamaleya Center and Sputnik V vaccine.

1. «Replicant adenovirus detected in all the Sputnik vaccine's batches presented to component II (Ad5): probable recombination. Component I (Ad26) it is not evaluated regarding replicant adenovirus» (Presentation from ANVISA public meeting on April 26, 2021).

   «The data we evaluated shows the presence of replicating virus» (Gustavo Mendes, General manager of medicines and biological products at ANVISA during the press conference on April 29, 2021).

Gamaleya Center's Response

- ANVISA could not conduct tests of the Sputnik V batches for the presence of RCA because no vials were ever provided to ANVISA. At its press conference on April 29, ANVISA corrected its position by saying it had not carried out its own testing and did not find any RCA, but was concerned about the Russian theoretical regulatory limit for that parameter.
- In fact, the Gamaleya Center had communicated that no RCA had ever been detected in Sputnik V vaccine to ANVISA before in its official letter dated March 26, 2021, which says clearly that «In addition we would like to inform you that during the release of the vaccine product at the Center site and at the contract site of Generium, not a single batch containing RCA was recorded» (please see the letter in Appendix # 1).
- Existing double quality controls carried out by Gamaleya Center and by the National State Laboratory of the Russian Federation (Roszdravnadzor, roszdravnadzor.gov.ru, an independent institution like ANVISA) have not detected any RCA in Sputnik V vaccine (please see the respective letter from Roszdravnadzor in Appendix # 2). Control for RCA is carried out not only for the finished product but also at different stages of production, including the viral seed and vaccine concentrate. Going forward the same double quality control procedures will continue to ensure that Sputnik V consists only of batches where RCA is not detected.
- Appendix # 3 contains the batch protocols confirming that no RCA has been detected in Sputnik V vaccine. For avoidance of doubt, column «Number of replication-competent adenoviruses per dose», section 4 «Control testing results» of batch protocols refers to a theoretical limit, while the actual results of RCA testing are provided in column «Average number of plaques in two iterations».
- Science magazine (https://www.sciencemag.org/news/2021/04/russias-covid-19-vaccine-safe-brazils-veto-sputnik-v-sparks-lawsuit-threat-and) article sheds light on the source of ANVISA's misunderstanding:

- The article quotes Jorge Kalil's correct understanding that strict double quality controls of both Gamaleya Institute and Roszdravnadzor confirmed that no RCAs were detected. «Immunologist Jorge Kalil, a vaccine expert at the Federal University of São Paulo, São Paulo, and member of the Data and Safety Monitoring Board at the U.S. National Institutes of Health, disagrees with ANVISA's interpretation. He believes Russian quality-control documents are actually referring to the sensitivity of the testing». And this is correct and consistent with the letter dated March 26th to ANVISA that confirms no RCA detected. Quality controls in Russia that show «less than quality control threshold» means «no detection».
- In the same article, Gustavo Mendes shows his incorrect understanding of Gamaleya reports. «Mendes told Science this is not the case and that if it were, Gamaleya would have reported «no detection». This statement is incorrect and highly unprofessional as shows that Mendes did not even check with Gamaleya Institute the meaning of the reports prior to drawing his incorrect conclusions and taking them to the public eliciting significant damage based on wrong interpretation.

- The existing norms for determining amounts of RCA in the Sputnik V vaccine are in full compliance with the FDA recommendations. Under the existing FDA regulations, from 33 RCA (based on an intratumoral dose of 10 ^ 12 particles) to 1000 RCA (based on a maximum intravenous dose of 3x10 ^ 13 particles) can enter the human body.

2. <u>«E1 is not deleted in Sputnik V vaccine».</u>

Gamaleya Center's Response

- The production technology begins with cloning the target gene (in this case, the S gene) into a plasmid with a fragment of the Ad5 genome already without the E1 region. That is, the E1 region does not exist initially, and the target genes are cloned into the site of its deletion. The technology of E1 deleted vectors has been

known for twenty years, its very basis does not allow «forgetting» the removal of E1. It is impossible to forget to delete something that does not originally exist in the technology.

Documentation provided to ANVISA clearly states that E1 has been deleted from Sputnik V. For instance, Pharmaceutical Development Document shows the following:

Table 2. Results of whole genome sequencing of 1-10 passages of rAd5-S-CoV2

Passage	1	2	3	4	5	6	7	8	9	10
E1 region genes	-	-	-	-	-	-	-	-	-	-

3. During the press conference on April 29, Mr. Mendes showed a video of the online meeting happened on March 23 between officials from ANVISA and the vaccine's developer:

ANVISA question: *I think I did it before but anyway we need to understand: once you have detected the RCA´s occurrence in your production, why you haven't gone back in development and chosen another cell substrate that had plasmids constructed in a way that there is very few homology sides and that generally they don´t allow a recombination between adenovirus and the cells? And when you considered continuing this development, what was the risk assessment conducted to really assess the risk of the presence of these particles in the vaccine? Because it's going to be administered in healthy people. So, what´s the justification that you have to proceed with this development for a vaccine that will be generally used in healthy people? This is something that should be addressed in this risk assessment and we want to know details about it.*

Gamaleya response (translated from Russian): 12:00 *"They said that they have used for the vaccine production a characterized cell lineage that can have its flaws and you are obviously right in stating that we could have gone back, taken a step back and begun using a different substance, but they are saying that this process would have*

taken much time. So, they chose to use the same substance they have been using since the beginning…"

Gamaleya Center's Response

The video clip in question was heavily edited and in fact it does not confirm Gamaleya Center's consent with RCA existence in Sputnik V vaccine. Response of Gamaleya Center in the video clip referred to the fundamental topics of vaccine development using HEK293 cells. To clarify the position of Gamaleya Center regarding RCAs existence in Sputnik V vaccine and avoid any misunderstanding, we sent the letter dated March 26, 2021 stating that there are no RCAs in any batches of Sputnik V vaccine produced.

We please ask ANVISA to confirm that it has in this letter, as well as in earlier correspondence (including the letter dated March 26 (Appendix # 1), the letter with Gamaleya Center's responses to ANVISA dated April 29), clearly received both Gamaleya Center's position and that of an independent Russian regulator Roszdravnadzor that through extensive quality control no RCA has been detected in Sputnik V vaccine. Please also acknowledge that ANVISA statements on this matter are based on incorrect assumptions as outlined in this letter and with supporting documents. We believe that such statements could have been avoided if ANVISA followed a standard procedure and verified its assumptions and conclusions with Gamaleya Center prior to making any public statements.

Alexander Vasilievich Pronin

Deputy Director of The Federal State Budgetary Institution N. F. Gamaleya National Research Center of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation

Appendix № 1

Official letter from Gamaleya dated March 26, 2021.

MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

Federal State Budgetary Institution
N. F. Gamaleya National Research Center of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation
(FSBI N.F. Gamaleya NRCEM of the Ministry of Health of Russia)

123098, Moscow, Gamaleya str., 18

Tel.: 8 499-193-30-01
Fax: 8 499-193-61-83

http://www.gamaleya.org
E-mail: info@gamaleya.org

26.03.2021 No.

On No. _____ of _____

TO WHOM IT MAY CONCERN

The Federal State Budgetary Institution N. F. Gamaleya National Research Center of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation informs that the RCA (Replication-competent adenovirus) tolerance standard for the Gam-Covid-Vac vaccine is no more than 100 RCA per dose. At the same time, this value has been agreed by the Russian regulatory authorities and the safety of the vaccine preparation, with such a tolerance value according to RCA, is confirmed by conducted preclinical and clinical studies, and is also confirmed in the course of mass vaccination of the population. In addition, we would like to inform you that during the release of the vaccine product at the Center site and at the contract site of JSC Generium, not a single batch containing RCA was recorded.

Professor Alexander Leonidovich Gintsburg

Director of the National Research Center for Epidemiology and Microbiology named after Honorary Academician N.F. Gamaleya of the Ministry of Health of the Russian Federation

Appendix № 2

Official letters from Ministry of Health and Roszdravnadzor.

Министерство здравоохранения
Российской Федерации

ФЕДЕРАЛЬНАЯ СЛУЖБА ПО НАДЗОРУ
В СФЕРЕ ЗДРАВООХРАНЕНИЯ
(РОСЗДРАВНАДЗОР)

Славянская пл. 4, стр. 1, Москва, 109074
Телефон: (495) 698 45 38; 698 15 74

03.05.2021 № 02-24306/21

На № ВЧ-1и/05.21 от 03.05.2021

О рассмотрении обращения

ООО «Вакцина человека»

Пресненская набережная, д. 8,
стр. 1, этаж 7, пом. I,
г. Москва, 123112

Федеральная служба по надзору в сфере здравоохранения в рамках компетенции рассмотрела обращение ООО «Вакцина человека», касающееся проведения контроля качества лекарственного препарата «Гам-КОВИД-Вак Комбинированная векторная вакцина для профилактики коронавирусной инфекции, вызываемой вирусом SARS-CoV-2» (далее – «Гам-КОВИД-Вак»), и сообщает.

В соответствии с правилами выдачи разрешения на ввод в гражданский оборот серии или партии иммунобиологического лекарственного препарата, выдачи заключения о соответствии серии или партии иммунобиологического лекарственного препарата требованиям, установленным при его государственной регистрации, утвержденными постановлением Правительства Российской Федерации от 26 ноября 2019 года N 1510 «О порядке ввода в гражданский оборот лекарственных препаратов для медицинского применения», ФГБУ «Информационно-методический центр по экспертизе, учету и анализу обращения средств медицинского применения» Росздравнадзора проводит испытания качества иммунобиологических лекарственных препаратов.

По результатам испытаний по показателю «Специфическая безопасность» методом «Реакция бляшкообразования на культуре клеток А549» ста сорока пяти серий второго компонента лекарственного препарата «Гам-КОВИД-Вак», введенных в гражданский оборот на территории Российской Федерации, репликативно-компетентных аденовирусов выявлено не было.

Дополнительно информируем, что 30.04.2021 Минздравом России утверждено изменение в документы, содержащиеся в регистрационном досье на зарегистрированный лекарственный препарат «Гам-КОВИД-Вак», согласно которому показатель «Специфическая безопасность» не превышает 50 репликативно-компетентных аденовирусов на дозу.

Врио руководителя Д.В. Пархоменко

Кудрявцева Е.М.
8 (499) 578 06 65

Appendix № 3

Selected three batch protocols, which confirm absence of any RCAs in all batches.

Gam-COVID-Vac. Control Testing Parameter Specific Safety COMPONENT II

1. Control testing date _08____January___ 2021

2. Information about Test Material

2.1. Background:

Item No.	Batch No.	Manufactured by	Intended use	Quantity to be tested
1	II-011220	CJSC LEKKO	Release control	3 samples
2	II -010121	CJSC LEKKO	Release control	3 samples
3	II -020120	CJSC LEKKO	Release control	3 samples
4	II -030121	CJSC LEKKO	Release control	3 samples
5	II -040120	CJSC LEKKO	Release control	3 samples
6	ZB00121	GENERIUM JSC	Release control	3 samples
7	ZB00221	GENERIUM JSC	Release control	3 samples
8	487031220	BIOCAD	Release control	3 samples
9				
10				

2.2. Storage conditions

According to regulatory documentation	Actual storage conditions	Compliance
Max -18 °C	*-20°C*	Yes / No
2 to 8 °C		Yes / No

3. Control testing protocol

3.1 Control testing equipment

Equipment	Serial No.	Equipment status Use is approved
CO_2 incubator	200260003	Yes / No
Laminar box class II / type A2	221.120.00.3652	Yes / No
Variable volume 20-200 mL dosimeter.	39282545	Yes / No
Variable volume 100-1000 mL dosimeter.	39382220	Yes / No

3.2 Reagents and auxiliary materials used in control testing

Reagent / Auxillary material	Ref. no. / OL number/record No.	Batch number	Use before
Cultural dishes 60 mm with A549 cell monolayer (10 pcs)	*	Passage No. *15*	*
Cultural medium DMEM 1x (serumless)	*No. 128*	-	*14.01.21*
Cover cultural medium DMEM 2x (OVER) (for A549)	*No. 129*	-	*14.01.21*
Cover 2% agar solution	*No. 130*	-	*14.01.21*
Neutral red dye 100x	*No. 97*	-	*06.04.21*
Positive control sample Ad5-RCA, titer *3.0 · 10^9* PFU*/mL*	-	*040320*	*03.2021*
serological pipettes, 5 mL, sterile, graduated, individually packaged	-	*27520061*	*01.10.2023*
serological pipettes, 10 mL, sterile, graduated, individually packaged	-	*0329004*	*28.10.2023*
Falcon tubes, 50 mL	-	*J1922390*	*28.09.2025*
Eppendorf tubes, 1.5 mL	-	*J191482M*	*28.07.2028*

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3.3 Preparation of the box for work

Box preparation control	Cleaning is completed	Yes / No
	UV treatment is completed	Yes / No

3.4 Reagents preparation

Item No.	SOP requirements	Requirement is met
1	Heat cultural medium DMEM 1x (serumless), cover medium DMEM 2x (OVER) to (37.0±1.0)°C	Yes / No
2	Melt the cover 2% agar solution	Yes / No
3	Prepare 1.5 mL tubes	Yes / No

3.5 Procedure

Item No.	SOP requirements	Requirement is met
1	Cell monolayer in the cultural dishes must be 70 to 90% (homogenous, without 'holes' and cell aggregates)	Yes / No
3.5.1	**Preparing a number of 10x dilutions of control samples**	
1	For each batch analyzed, allow vials with test material to stand at room temperature. Combine the contents.	Yes / No
2	Prepare eight 1.5 mL tubes for each test sample, label each two tubes as follows: (10^{-1}) dilution; (10^{-2}) dilution; (10^{-3}) dilution; (10^{-4}) dilution	Yes / No
3	Prepare four 10x dilutions of each test sample in two iterations from 10^{-1} to 10^{-4} dilution (150 µl of sample +1350 µl of serumless medium)	Yes / No
3.5.2	**Preparing a positive control sample**	
1	Prepare as many 1.5 mL tubes as correspond to the PCS titer order. Label accordingly.	Yes / No
2	Prepare 10x dilutions of PCS from 10^{-1} to 10^{-9} (first dilution: 20 µl of PCS + 180 µl of serumless medium; then - 150 µl of sample + 1350 µl of serumless medium)	Yes / No
3.5.3	**Transferring dilutions (of PCS and test samples) to cultural dishes**	
1	Label cultural dishes with a cell monolayer as follows: C- - monolayer control PCS – 10^{-7} PCS – 10^{-8} PCS - 10^{-9} For each test sample: Experiment 10^{-2} (two dishes) Experiment 10^{-3} (two dishes) Experiment 10^{-4} (two dishes)	Yes / No
2	Add 1 ml of serumless growth medium to monolayer control dish (negative control)	Yes / No
3	Add test sample starting from the largest dilution. Stir the contents of the (dilution 10^{-4}) tube from first iteration, add 1 mL to cultural dish. Similarly, add second iteration dilution.	Yes / No
4	Similarly, add dilutions 10^{-3} and 10^{-2}.	Yes / No
5	Similarly, add each test sample as per items 3-4	Yes / No
6	Stir the contents of the (dilution 10^{-9}) tube, add 1 mL to cultural dish. Similarly, add dilutions 10^{-8} and 10^{-7}.	Yes / No

Gam-COVID-Vac. Control Testing Parameter Specific Safety COMPONENT II

Item No.	SOP requirements	Requirement is met
7	Incubate the dishes in a CO_2 incubator at (37±1) ° C in an atmosphere with (5.0±0.5)% CO_2 for 4–6 hours. Begins *12*[10]; Ends *17*[20]	Yes / No
8	Prepare a cover medium. For 10 dishes: add 25 mL of cover cultural medium DMEM 2x (OVER) and 25 mL of 2 % agar solution to a sterile 50 mL tube. Carefully mix he contents with a pipette avoiding to create bubbles.	Yes / No
9	Remove the dilution medium from the dishes. Transfer the cover medium to the dishes as follows: dishes with negative control → dishes with dilutions of test samples, beginning with the lowest dilution → dishes with positive control. Pour slowly, along the wall, 5 mL of cover medium into each dish.	Yes / No
10	Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 3 days.	Yes / No
Executive	[signature]	*Goldovskaya P.P.*
Date: *08.01.2021*		
11	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 3 days.	Yes / No
Executive	[signature]	*Goldovskaya P.P.*
Date: *11.01.2021*		
12	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 2-3 days.	Yes / No
Executive	[signature]	*Goldovskaya P.P.*
Date: *14.01.2021*		
3.5.4	**Staining cell monolayer with neutral red dye**	
1	Prepare dyed cover nutritional medium. Mix in a 50 mL tube 15 ml of cover medium DMEM 2x, 13.2 mL of 2 % agar solution, and 1.8 mL of neutral red dye solution.	Yes / No
2	Add 3 mL of dyed cover medium to each dish	Yes / No
3	Incubate the dishes in a CO_2 incubator at (37±1) ° C with (5.0±0.5)% CO_2 for 24 hours. Results are to be registered on days 9-10.	Yes / No
Executive	[signature]	*Goldovskaya P.P.*
Date: *17.01.2021*		

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Gam-COVID-Vac. Control Testing Parameter Specific Safety COMPONENT II

Item No.	SOP requirements		Requirement is met
3.5.5	**Recording the results**		
1	The control results are to be recorded, provided there is no degeneration of the cell monolayer in the Petri dish with negative control.		
2	When staining with neutral red the live cell monolayer turns reddish, while plaques appear as unstained round spots. Plaques are to be counted visually in those dilutions of the samples where they do not merge with one another. Enter the results in Table 1.1.		
3	Control testing results are to be recorded if only individual plaques are observable in PCS dishes with 10^{-9} dilution, with their number increasing pro rata in subsequent dilutions (10^{-8} and 10^{-7}).		
3.5.6	**Calculation procedure** (Excel may be used for calculations)		
1	Determine the average number of plaques (in 2 dishes) for each dilution.	$n_i av = n_{i\,1} + n_{i\,2}$	
2	Determine the number of recombinant adenoviral particles (RCA) for each dilution in which plaques were detected	where $Ni = [(n_i av \times 10^a)/V] \times 0.5$, where $n_i av$ – the average number of plaque areas in 2 dishes; a – drug dilution, mL; V - volume of solution added per dish, mL; 0.5 - volume of a vaccine dose, mL.	
3	Calculate the arithmetic average of the results for each dilution	$N_{av} = (N_i + N_{i+1}) / 2$, where $N_i; N_{i+1}$ - number of RCA for each dilution 2 - number of iterations	

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4. Results of control testing

Results recording date: 18.01.2021

Table 1.1 Results recording.

Preparing the test sample	Number of plaques		Average number of plaques in two iterations	Number of replication-competent adenoviruses per dose
	1st cultural dish	2nd cultural dish		
Negative control		-	-	-
Positive control sample				
Dilution 10^{-7}	*>200*	-		
Dilution 10^{-8}	*16*	-		
Dilution 10^{-9}	*2*	-		
1. Batch II-011220 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
2. Batch II-010120 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
3. Batch II-020120 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
4. Batch II-030120 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
5. Batch II-040120 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
6. Batch ZB00121 JSC GENERIUM				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
7. Batch ZB00221 JSC GENERIUM				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
8. Batch 487031220 BIOCAD				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		

Estimate

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Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II.

1. Control testing date _11____January___ 2021

2. Information about Test Material

2.1. Background:

Item No.	Batch No.	Manufactured by	Intended use	Quantity to be tested
1	II- 10121	BINNOPHARM	Release control	3 samples
2	II- 20121	BINNOPHARM	Release control	3 samples
3	II-010121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
4	II-020121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
5	II-030121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
6	II-040121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
7	ZB00321	GENERIUM JSC	Release control	3 samples
8	ZB00421	GENERIUM JSC	Release control	3 samples
9	II-050120	CJSC LEKKO	Release control	3 samples
10	II-060121	CJSC LEKKO	Release control	3 samples

2.2. Storage conditions

According to regulatory documentation	Actual storage conditions	Compliance
Max -18 °C	*- 20°C*	Yes/ No
2 to 8 °C	-	Yes/ No

3. Control testing protocol

3.1 Control testing equipment

Equipment	Serial No.	Equipment status Use is approved
CO_2 incubator	200260003	Yes/ No
Laminar flow unit class II / type A2	221.120.00.3652	Yes/ No
Variable volume 20-200 mL dosimeter.	39282545	Yes/ No
Variable volume 100-1000 mL dosimeter.	39382220	Yes/ No

3.2 Reagents and auxiliary materials used in control testing

Reagent/ auxiliary material	Ref. no. / OL number/record No.	Batch number	Shelf life
Cultural dishes 60 mm with A549 cell monolayer (10 pcs)	*	Passage No. *16*	*
Cultural medium DMEM 1x (serumless)	*N 128*	–	*14.01.21*
Cover cultural medium DMEM 2x (OVER) (for A549)	*N 129*	–	*14.01.21*
Cover 2% agar solution	*N 130*	–	*14.01.21*
Neutral red dye 100x	*N 97*	–	*06.04.21*
Positive control sample Ad5-RCA, titer *3.0 10^9* PFU/*mL*	–	*040320*	*01.10.2023*
serological pipettes, 5 mL, sterile, graduated, individually packaged	–	*27520061*	*08.10.2023*
serological pipettes, 10 mL, sterile, graduated, individually packaged	–	*0032004*	*14.01.21*

Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II.

Falcon tubes, 50 mL	–	J1922390	28.09.25
Eppendorf tubes, 1.5 mL	–	J191482M	28.07.28

3.3 Preparation of the box for work

Box preparation control	Cleaning is completed	Yes/ No
	UV treatment is completed	Yes/ No

3.4 Reagents preparation

Item No.	SOP requirements	The requirement is met
1	Heat cultural medium DMEM 1x (serumless), cover medium DMEM 2x (OVER) to (37.0±1.0)°C	Yes/ No
2	Melt the cover 2% agar solution	Yes/ No
3	Prepare 1.5 mL tubes	Yes/ No

3.5 Procedure

Item No.	SOP requirements	The requirement is met
1	Cell monolayer in the cultural dishes must be 70 to 90% (homogenous, without 'holes' and cell aggregates)	Yes/ No
3.5.1 Preparing a number of 10x dilutions of control samples		
1	For each batch analyzed, allow vials with test material to stand at room temperature. Combine the contents.	Yes/ No
2	Prepare eight 1.5 mL tubes for each test sample, label each two tubes as follows: (10^{-1}) dilution; (10^{-2}) dilution; (10^{-3}) dilution; (10^{-4}) dilution	Yes/ No
3	Prepare four 10x dilutions of each test sample in two iterations from 10^{-1} to 10^{-4} dilution (150 µl of sample +1350 µl of serumless medium)	Yes/ No
3.5.2 Preparing a positive control sample		
1	Prepare as many 1.5 mL tubes as correspond to the PCS titer order. Label accordingly.	Yes/ No
2	Prepare 10x dilutions of PCS from 10^{-1} to 10^{-9} (first dilution: 20 µl of PCS + 180 µl of serumless medium; then - 150 µl of sample + 1350 µl of serumless medium)	Yes/ No
3.5.3 Transferring dilutions (of PCS and test samples) to cultural dishes		
1	Label cultural dishes with a cell monolayer as follows: C- - monolayer control PCS – 10^{-7} PCS – 10^{-8} PCS – 10^{-9} For each test sample: Experiment 10^{-2} (two dishes) Experiment 10^{-3} (two dishes) Experiment 10^{-4} (two dishes)	Yes/ No
2	Add 1 ml of serumless growth medium to monolayer control dish (negative control)	Yes/ No

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Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II.

Item No.	SOP requirements	The requirement is met
3	Add test sample starting from the largest dilution. Stir the contents of the (dilution 10^{-4}) tube from first iteration, add 1 mL to cultural dish. Similarly, add second iteration dilution.	Yes/ No
4	Similarly, add dilutions 10^{-3} and 10^{-2}.	Yes/ No
5	Similarly, add each test sample as per items 3-4	Yes/ No
6	Stir the contents of the (dilution 10^{-9}) tube, add 1 mL to cultural dish. Similarly, add dilutions 10^{-8} and 10^{-7}.	Yes/ No
7	Incubate the dishes in a CO_2 incubator at (37±1) ° C with (5.0±0.5)% CO_2 for 4-6 hours. Begins *1135*; Ends *16*[15]	Yes/ No
8	Prepare a cover medium. For 10 dishes: add 25 mL of cover cultural medium DMEM 2x (OVER) and 25 mL of 2 % agar solution to a sterile 50 mL tube. Carefully mix he contents with a pipette avoiding to create bubbles.	Yes/ No
9	Remove the dilution medium from the dishes. Transfer the cover medium to the dishes as follows: dishes with negative control → dishes with dilutions of test samples, beginning with the lowest dilution → dishes with positive control. Pour slowly, along the wall, 5 mL of cover medium into each dish.	Yes/ No
10	Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 3 days.	Yes/ No
Executive	[signature]	*Goldovskaya P.P.*
Date *11.01.2021*		
11	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 3 days.	Yes/ No
Executive	[signature]	*Goldovskaya P.P.*
Date *14.01.2021*		
12	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 2-3 days.	Yes/ No
Executive	[signature]	*Goldovskaya P.P.*
Date *17.01.2021*		
3.5.4 Staining monolayer of cells with neutral red dye		
1	Prepare dyed cover nutritional medium. Mix in a 50 mL tube 15 ml of cover medium DMEM 2x, 13.2 mL of 2 % agar solution, and 1.8 mL of neutral red dye solution.	Yes/ No
2	Add 3 mL of dyed cover medium to each dish	Yes/ No
3	Incubate the dishes in a CO_2 incubator at (37±1) ° C with (5.0±0.5)% of CO_2 for 24 hours. Results are to be registered on days 9-10.	Yes/ No
Executive	[signature]	*Goldovskaya P.P.*

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Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II.

Item No.	SOP requirements	The requirement is met
Date *20.01.2021*		
3.5.5 Recording the results		
1	The control results are to be recorded, provided there is no degeneration of the cell monolayer in the Petri dish with negative control.	
2	When staining with neutral red the live cell monolayer turns reddish, while plaques appear as unstained round spots. Plaques are to be counted visually in those dilutions of the samples where they do not merge with one another. Enter the results in Table 1.1.	
3	Control testing results are to be recorded if only individual plaques are observable in PCS dishes with 10^{-9} dilution, with their number increasing pro rata in subsequent dilutions (10^{-8} and 10^{-7}).	
3.5.6 Calculation procedure (Excel may be used for calculations)		
1	Determine the average number of plaques (in 2 dishes) for each dilution.	$n_i av = n_{i\,1} + n_{i\,2}$
2	Determine the number of recombinant adenoviral particles (RCA) for each dilution in which plaques were detected	where $Ni = [(n_i av \times 10^a)/V] \times 0.5$, where $n_i av$ - average number in 2 dishes; a - drug dilution, mL; V - volume of solution added per dish, mL; 0.5 - volume of a vaccine dose, mL.
3	Calculate the arithmetic average of the results for each dilution	$N_{av} = (N_i + N_{i+1}) / 2$, where $N_i; N_{i+1}$ - number of RCA for each dilution 2 - Number of iterations

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4. Control testing results

Results recording date 21.01.2021

Table 1.1 Results recording

Preparing the test sample	Number of plaques		Average number of plaques in two iterations	Number of replication-competent adenoviruses per dose
	1st cultural dish	2nd cultural dish		
Negative control		-	-	-
Positive control sample				
Dilution 10^{-7}	*>200*	–	–	–
Dilution 10^{-8}	*12*	–		
Dilution 10^{-9}	*1*	–		
1. Batch: II-10121 BINNOPHARM				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
2. Batch: II-20121 BINNOPHARM				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
3. Batch II-010120 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
4. Batch II-020120 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
5. Batch II-030120 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
6. Batch II-040120 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
7. Batch ZB00321 GENERIUM JSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
8. Batch ZB00421 GENERIUM JSC				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
9. **Batch II-050121 CJSC LEKKO**				
Dilution 10^{-2}	*0*	*0*	*0*	Less than 50 / dose
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		
10. Batch II-060121 CJSC LEKKO				

Dilution 10^{-2}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-3}	*0*	*0*		
Dilution 10^{-4}	*0*	*0*		

Estimate

Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II

1. Control testing date _18____January___ 2021

2. Information about Test Material

2.1. Background:

Item No.	Batch No.	Manufactured by	Intended use	Quantity to be tested
1	II-050121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
2	II-060121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
3	II-070121	Pharmstandard-UfaVITA OJSC	Release control	3 samples
4	II- 30121	BINNOPHARM	Release control	3 samples
5	ZB00521	GENERIUM JSC	Release control	3 samples
6	II-070121	CJSC LEKKO	Release control	3 samples
7				
8				
9				
10				

2.2. Storage conditions

According to regulatory documentation	Actual storage conditions	Compliance
Max -18 °C	*-20°C*	Yes / No
2 to 8 °C	-	Yes / No

3. Control testing protocol

3.1 Control testing equipment

Equipment	Serial No.	Equipment status Use is approved
CO_2 incubator	200260003	Yes / No
Laminar box class II / type A2	221.120.00.3652	Yes / No
Variable volume 20-200 mL dosimeter.	39282545	Yes / No
Variable volume 100-1000 mL dosimeter.	39382220	Yes / No

3.2 Reagents and auxiliary materials used in control testing

Reagent / Auxillary material	Ref. no. / OL number/record No.	Batch number	Shelf life
Cultural dishes 60 mm with A549 cell monolayer (10 pcs)	*	Passage No. *18*	*
Cultural medium DMEM 1x (serumless)	*N°2*	-	*11.02.21*
Cover cultural medium DMEM 2x (OVER) (for A549)	*N°4*	-	*11.02.21*
Cover 2% agar solution	*N°5*	-	*11.02.21*
Neutral red dye 100x	*N°97*	-	*06.04.21*
Positive control sample Ad5-RCA, titer $3.0 \cdot 10^9$ PFU/*mL*	-	*040320*	*03.2021*
serological pipettes, 5 mL, sterile, graduated, individually packaged	-	*27520061*	*01.10.23*
serological pipettes, 10 mL, sterile, graduated, individually packaged	-	*0329004*	*28.10.23*
Falcon tubes, 50 mL	-	*J1922390*	*28.09.25*
Eppendorf tubes, 1.5 mL	-	*J191482M*	*28.07.28*

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3.3 Preparation of the box for work

Box preparation control	Cleaning is completed	Yes / No
	UV treatment is completed	Yes / No

3.4 Reagents preparation

Item No.	SOP requirements	The requirement is met
1	Heat cultural medium DMEM 1x (serumless), cover medium DMEM 2x (OVER) to (37.0±1.0)°C	Yes / No
2	Melt the cover 2% agar solution	Yes / No
3	Prepare 1.5 mL tubes	Yes / No

3.5 Procedure

Item No.	SOP requirements	The requirement is met
1	Cell monolayer in the cultural dishes must be 70 to 90% (homogenous, without 'holes' and cell aggregates)	Yes / No
3.5.1	**Preparing a number of 10x dilutions of control samples**	
1	For each batch analyzed, allow vials with test material to stand at room temperature. Combine the contents.	Yes / No
2	Prepare eight 1.5 mL tubes for each test sample, label each two tubes as follows: (10^{-1}) dilution; (10^{-2}) dilution; (10^{-3}) dilution; (10^{-4}) dilution	Yes / No
3	Prepare four 10x dilutions of each test sample in two iterations from 10^{-1} to 10^{-4} dilution (150 µl of sample +1350 µl of serumless medium)	Yes / No
3.5.2	**Preparing a positive control sample**	
1	Prepare as many 1.5 mL tubes as correspond to the PCS titer order. Label accordingly.	Yes / No
2	Prepare 10x dilutions of PCS from 10^{-1} to 10^{-9} (first dilution: 20 µl of PCS + 180 µl of serumless medium; then - 150 µl of sample + 1350 µl of serumless medium)	Yes / No
3.5.3	**Transferring dilutions (of PCS and test samples) to cultural dishes**	
1	Label cultural dishes with a cell monolayer as follows: C- - monolayer control PCS – 10^{-7} PCS – 10^{-8} PCS - 10^{-9} For each test sample: Experiment 10^{-2} (two dishes) Experiment 10^{-3} (two dishes) Experiment 10^{-4} (two dishes)	Yes / No
2	Add 1 ml of serumless growth medium to monolayer control dish (negative control)	Yes / No
3	Add test sample starting from the largest dilution. Stir the contents of the (dilution 10^{-4}) tube from first iteration, add 1 mL to cultural dish. Similarly, add second iteration dilution.	Yes / No
4	Similarly, add dilutions 10^{-3} and 10^{-2}.	Yes / No
5	Similarly, add each test sample as per items 3-4	Yes / No
6	Stir the contents of the (dilution 10^{-9}) tube, add 1 mL to cultural dish. Similarly, add dilutions 10^{-8} and 10^{-7}.	Yes / No

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Item No.	SOP requirements	The requirement is met
7	Incubate the dishes in a CO_2 incubator at (37±1) ° C in an atmosphere with (5.0±0.5)% CO_2 for 4–6 hours. Begins *12*^{00}; Ends *17*^{00}	(Yes) / No
8	Prepare a cover medium. For 10 dishes: add 25 mL of cover cultural medium DMEM 2x (OVER) and 25 mL of 2 % agar solution to a sterile 50 mL tube. Carefully mix he contents with a pipette avoiding to create bubbles.	(Yes) / No
9	Remove the dilution medium from the dishes. Transfer the cover medium to the dishes as follows: dishes with negative control → dishes with dilutions of test samples, beginning with the lowest dilution → dishes with positive control. Pour slowly, along the wall, 5 mL of cover medium into each dish.	(Yes) / No
10	Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5) % CO_2 for 3 days.	(Yes) / No
Executive	[signature]	*Goldovskaya P.P.*
Data *18.01.2021*		
11	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5)% of CO_2 for 3 days.	(Yes) / No
Executive	[signature]	*Goldovskaya P.P.*
Date *21.01.2021*		
12	Add 5 mL of medium each to the dishes according to items 7-8. Incubate the dishes in a CO_2 incubator at (37.0±1.0) °C with (5.0±0.5)% of CO_2 for 2-3 days.	(Yes) / No
Executive	[signature]	*Goldovskaya P.P.*
Date *24.01.2021*		
3.5.4	**Staining monolayer of cells with neutral red dye**	
1	Prepare dyed cover nutritional medium. Mix in a 50 mL tube 15 ml of cover medium DMEM 2x, 13.2 mL of 2 % agar solution, and 1.8 mL of neutral red dye solution.	(Yes) / No
2	Add 3 mL of dyed cover medium to each dish	(Yes) / No
3	Incubate the dishes in a CO_2 incubator at (37±1) ° C with (5.0±0.5)% CO_2 for 24 hours. Results are to be registered on days 9-10.	(Yes) / No
Executive	[signature]	*Goldovskaya P.P.*
Date *27.01.2021*		

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Gam-COVID-Vac. Control Testing Parameter Specific Safety Component II

Item No.	SOP requirements	The requirement is met
3.5.5	**Recording the results**	
1	The control results are to be recorded, provided there is no degeneration of the cell monolayer in the Petri dish with negative control.	
2	When staining with neutral red the live cell monolayer turns reddish, while plaques appear as unstained round spots. Plaques are to be counted visually in those dilutions of the samples where they do not merge with one another. Enter the results in Table 1.1.	
3	Control testing results are to be recorded if only individual plaques are observable in PCS dishes with 10^{-9} dilution, with their number increasing pro rata in subsequent dilutions (10^{-8} and 10^{-7}).	
3.5.6	**Calculation procedure** (Excel may be used for calculations)	
1	Determine the average number of plaques (in 2 dishes) for each dilution.	$n_i av = n_{i\,1} + n_{i\,2}$
2	Determine the number of recombinant adenoviral particles (RCA) for each dilution in which plaques were detected	where $Ni = [(n_i av \times 10^a)/V] \times 0.5$, where $n_i av$ – the average number of plaque areas in 2 dishes; a – drug dilution, mL; V - volume of solution added per dish, mL; 0.5 - volume of a vaccine dose, mL.
3	Calculate the arithmetic average of the results for each dilution	$N_{av} = (N_i + N_{i+1}) / 2$, where $N_i; N_{i+1}$ - number of RCA for each dilution 2 - number of iterations

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4. Results of control work

Results recording date: 28.01.2021

Table 1.1 Results recording

Preparing the test sample	Number of plaques		Average number of plaques in two iterations	Number of replication-competent adenoviruses per dose
	1st cultural dish	2nd cultural dish		
Negative control		-	-	-
Positive control sample				
Dilution 10^{-7}	*>200*	-		
Dilution 10^{-8}	*20*	-		
Dilution 10^{-9}	*2*	-		
1. Batch II-050121 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
2. Batch II-060121 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
3. Batch II-070121 Pharmstandard-UfaVITA OJSC				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
4. Batch II-30120 BINNOPHARM				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
5. Batch ZB00521 JSC GENERIUM				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		
6. Batch II-040120 CJSC LEKKO				
Dilution 10^{-2}	*0*	*0*		
Dilution 10^{-3}	*0*	*0*	*0*	*Less than 50 / dose*
Dilution 10^{-4}	*0*	*0*		

Estimate

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