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Preparation and transfer of *Burkholderia mallei* production strain 5584 in accordance with the biosafety requirements

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SUMMARY

The Veterinary Service of the Russian Federation takes measures to ensure regular control of livestock health status, to prevent infectious diseases and their introduction into the country; and if such diseases are diagnosed, it takes measures to prevent their spread and contain outbreaks as soon as possible. Success of the taken measures depends on the use of various diagnostic, preventive and therapeutic drugs. In order to produce such medicinal products, biofactories use production and reference strains with stable biological properties, which are stored in national collections of microorganisms. The only keeper of glanders strains is the Laboratory for Collection of Strains of Microorganisms in the FSBSI «FCTRBS-ARRVI», subordinated to the Ministry of Agriculture of the Russian Federation. The following steps were taken due to the official request from FKP Kursk Biofactory – BIOK Company for the transfer of *Burkholderia mallei* production strain 5584 from the collection of the institution: the strain was passaged in golden hamsters, its viability was determined and biological properties of the culture were studied. The strain was transferred in accordance with the established procedure and in compliance with the biosafety requirements. As the work progressed, *Burkholderia mallei* strain 5584 culture was isolated and freeze-dried. Before the transfer, biological properties of the freeze-dried *Burkholderia mallei* strain 5584 were studied for their compliance with the passport data. The obtained results showed that the Laboratory for Collection of Strains of Microorganisms in the FSBSI «FCTRBS-ARRVI» provides optimal conditions to preserve the strain viability and initial biological properties after 5 years of storage. Analysis of the data obtained during the transfer of *Burkholderia mallei* strain 5584 allowed us to assess the actions taken at all stages of the procedure. It was established that the transfer procedure for the requested glanders production strain complied with the biosafety requirements and regulatory framework regulating the process.

Keywords: *Burkholderia mallei*, viability, biological properties, strain transfer

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Особенности подготовки и выдачи производственного штамма 5584 *Burkholderia mallei* в соответствии с требованиями биологической безопасности

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РЕЗЮМЕ

Ветеринарной службой Российской Федерации проводится комплекс мер, направленных на регулярный контроль состояния поголовья сельскохозяйственных животных, профилактику инфекционных заболеваний и недопущение их завоза в страну, а в случае диагностирования – предотвращение распространения и купирование в кратчайшие сроки. Успешная реализация данных мероприятий возможна благодаря использованию различных диагностических, профилактических и лечебных препаратов. С целью их получения предприятия биологической промышленности применяют производственные и эталонные штаммы микроорганизмов со стабильными биологическими свойствами, которые хранятся в государственных коллекциях микроорганизмов. Единственным держателем штаммов возбудителя сапа является лаборатория коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ», подведомственного Министерству сельского хозяйства Российской Федерации. В связи с официальным запросом ФКП «Курская биофабрика – фирма «БИОК» о получении производственного штамма 5584 *Burkholderia mallei* из коллекции учреждения были выполнены следующие работы: проведен пассаж штамма на золотистых хомячках, определена жизнеспособность и изучены биологические свойства культуры, в установленном порядке, с соблюдением требований биологической безопасности, произведена выдача штамма. В процессе проведенной работы выделена культура штамма 5584 *Burkholderia mallei*, которая была лиофилизирована. Перед выдачей были изучены биологические свойства лиофилизированного штамма 5584 *Burkholderia mallei* на соответствие их паспортным данным. Полученные результаты показали, что в лаборатории коллекции штаммов микроорганизмов ФГБНУ «ФЦТРБ-ВНИВИ» созданы оптимальные условия для сохранения его жизнеспособности и исходных биологических свойств по истечении 5 лет хранения. Анализ данных, полученных при выполнении работ по передаче штамма 5584 *Burkholderia mallei*, позволил дать оценку действий на всех ее этапах. Установлено, что порядок выдачи запрашиваемого производственного штамма возбудителя сапа соответствовал требованиям биологической безопасности и нормативно-правовым документам, регламентирующим проведенные мероприятия.

Ключевые слова: *Burkholderia mallei*, жизнеспособность, биологические свойства, передача штамма

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INTRODUCTION

Freedom of the Russian Federation from infectious diseases depends on a set of anti-epizootic measures that includes systematic monitoring of livestock health, prevention of infectious disease and spread; prevention of its introduction into the country, and if it is diagnosed – prevention of its spread and containment of the outbreak in the shortest possible time [1].

Due to the impact of globalization on spread of infections, regular emergence of new and old infectious diseases, total urbanization and new economic conditions, not a single country is now able to completely protect its territory from introduction of highly dangerous diseases. Such infections pose a threat not only to animals, but also to people and can damage national economies, break economic and cultural links [2].

Therefore, it is of top priority to develop and produce reliable, highly-specific diagnostic and preventive drugs, therapeutic agents and modern indication systems at the country's biofactories; such production is regulated by Instruction of the Chief Veterinary Officer of the Russian Federation No. 22-7/443 of 08.05.92 "Issuing a permit for production of veterinary medicinal products and their certification". In order to produce diagnostic preparations and vaccines, biofactories use production and reference strains from the national collections of pathogenic microorganisms, where optimal conditions are ensured to preserve them in the original state, excluding any transformation in biological, serological, toxicological properties and sensitivity to antibiotics [3–8]. Strains of highly dangerous microorganisms stored in the national collections are handled in accordance with the regulatory framework and the Federal laws and resolutions of the Government of the Russian Federation [8, 9].

Glanders is a dangerous bacterial infection from the group of zoonoses – a highly dangerous, highly contagious disease of animals and humans; presently, there is no vaccine or specific treatment available for glanders. As scientific publications demonstrate, there are cases of professional infection with the *Burkholderia mallei* bacterium among microbiologists working with this pathogen. As a result, burkholderia is equated according to its pathogenicity with such pathogens as *Yersinia pestis* and *Francisella tularensis*. Recently, several cases of *B. mallei* infection in humans have been reported in the laboratories of Russia and the USA, including one fatal outcome [10]. Currently, the Russian Federation is free from the disease, but the risk of introduction from other countries remains [2].

Taking into account the above, there is still a need to produce high-quality products for glanders diagnosis. FKP "Kursk Biofactory – BIOK Company" produces "Glanders serum for the complement fixation test", "Glanders antigen for the complement fixation test", "Stained glanders antigen for the plate agglutination test", as well as "Mallein". These medicinal products are made on the basis of *B. mallei* production strain 5584, received in accordance with the established procedure from the National (official) Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI", which is the only keeper of glanders strains among other institutions subordinate to the Ministry of Agriculture of the Russian Federation [2].

Following the official request from FKP "Kursk Biofactory – BIOK Company" to receive *Burkholderia mallei* production strain 5584 from the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI", the purpose of this work was to conduct a passage, determine viability, study biological properties and transfer the pathogen in accordance with the established procedure and in compliance with the biosafety requirements.

MATERIALS AND METHODS

The work was carried out in accordance with SP 1.3.3118-13 "Safety guide for work with Pathogenicity Groups I–II microorganisms". The freeze-dried *B. mallei* production strain 5584 was used. The strain was stored in the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI" at a temperature of +4 °C and standardized according to the international Master seed standard.

B. mallei strain 5584 was passaged in golden hamsters, when the pathogen suspension was administered subcutaneously into the occipital region at a dose of 5 and 10 IU according to the turbidity standard (GISK named after L. A. Tarasevich), in the volume of 0.2 and 0.4 ml, respectively. The dead hamsters, as well as those ones in agony, were euthanized by ether and subjected to autopsy. The work with laboratory animals was carried out in accordance with the Helsinki Convention on the Humane Treatment of Experimental Animals (1975) and the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes (1986).

Bacteria culture tests were carried out in liver, spleen, lungs, injection site, heart blood; the tested materials were inoculated using a Pasteur pipette on meat-peptone agar and broth with 4% glycerin (MPGA, MPGB) and incubated at 37 °C for 3–10 days. Virological properties of the isolated culture were tested to match the glanders pathogen. If the observed signs matched the passport data, the strain was freeze-dried.

Table
Biological properties of *Burkholderia mallei* strain 5584

Name of the strain	5584 *Burkholderia mallei*
Formation of hydrogen sulfide, mm	0.2
Curdling of skimmed milk	Milk curdling
Growth in potatoes according to Pavlovsky	In the first days, the colonies are in the form of honey droplets, then merge into a slimy plaque, on Day 7–8 the color turns to brown
Growth in MPGB	Turbidity of the medium, a delicate film on the surface, over time a slimy precipitate forms at the bottom of the test tube, which rises in the form of a corkscrew when shaken
Growth in MPGA	Growth in the form of translucent colonies with smooth edges and a mother-of-pearl hue
Gram Staining	Gram-negative rods with pronounced granular layer
Loeffler Staining	Pale blue rods with red granular layer
Motility	Non-motile
Proteolytic activity on 12% gelatin	Does not break down

Fig. 1. Materials required for packing vials with glanders production strain 5584

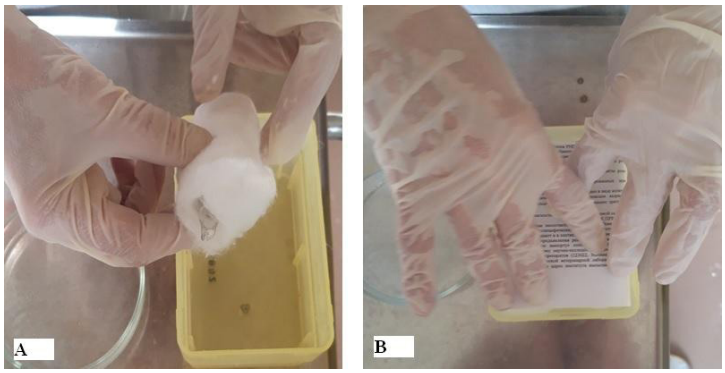

Fig. 2. Placing vials into containers and enclosing the first copies of the accompanying documents

Before FKP "Kursk Biofactory – BIOK Company" received the strain requested, viability of the culture was determined and its biological properties were controlled. For these purposes, a vial with a strain was taken from the collection, then appropriately registered in the "Biological agent movements records" (Form No. 514/u) and the "Individual registration card for the biological agent in the collection" (Form No. 517/u). The vial was opened, the freeze-dried mass was suspended in a 0.85% sodium chloride solution, and the resulting bacteria suspension was inoculated onto nutrient media containing 4% glycerin. The vial and the culture remains in it were destroyed, a report was made on opening a vial with biological agents of Pathogenicity Groups I–II for the purpose of inoculation or destruction (Form No. 521/u) and a mark was made in the "Journal of disinfection for biological agents" (Form No. 520/u). The cultures were incubated at 37 °C in a thermostat for 3–10 days. As soon as the second generation of the strain culture was obtained, its biological properties were tested to match the passport data. For this purpose, the following properties were controlled: cultural, microscopic (tinctorial-morphological and mobility), biochemical (saccharolytic, proteolytic).

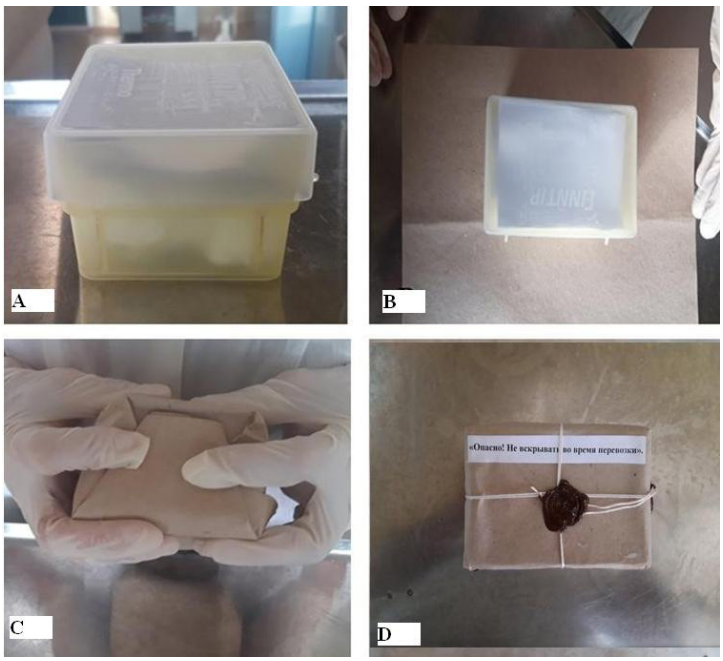

Fig. 3. Packing a container with a vial with B. mallei production strain 5584

RESULTS AND DISCUSSION

B. mallei production strain 5584 transfer procedure started after receipt of the following documents from FKP "Kursk Biofactory – BIOK Company": an official request signed by the director; photocopies of the license and hygiene certificate; bank details certifying safe working conditions are in place and the company is eligible to receive it.

B. mallei strain 5584 was passaged in golden hamsters and freeze-dried. Before FKP "Kursk Biofactory – BIOK Company" received the requested strain, its viability was tested according to the biological properties (Table), given in the passport and corresponding to the indicators given in the Bergey's Manual of Determinative Bacteriology (1980).

The conducted studies show that strain 5584, stored for 5 years in a freeze-dried form at a temperature of +4 °C, is viable. Biological properties were tested on differential diagnostic media and their compliance with the passport data was established. It suggests that the Laboratory for Collection of Strains of Microorganisms provides optimal storage and working conditions for the microorganisms.

The transfer of the glanders production strain is associated with potential contamination risks (during studies of viability and basic properties of the cultures, due to careless handling of vials when packing them, due to mistakes made while processing documents and due to other unauthorized actions, etc.), which is confirmed by recorded glanders cases among veterinary and medical staff working with pathogen cultures. Glanders aerosol cultures are especially hazardous due to the risk of inhalation [10]. As mentioned above, glanders production strain 5584 was transferred to FKP "Kursk Biofactory – BIOK Company" strictly in accordance with the requirements of SP 1.2.036-95 and SP 1.3._-17.

Based on the written permission of the FSBSI "FCTRBS-ARRVI" director *B. mallei* production strain 5584 (biological properties checked) was transferred to representatives of FKP "Kursk Biofactory – BIOK Company", following presentation of their ID documents and a power of attorney in accordance with a transfer certificate indicating the number of vials (Form No. 525/u) and a record made in the "Journal of biological agents transfer" (Form No. 516/u). In order to pack the vial with the strain, the following materials were prepared: a waterproof container with a hermetically sealed lid, hygroscopic cotton wool, a twine, wrapping paper, sealing wax (Fig. 1).

The following accompanying documents are issued for the contents of the container (in two copies): a letter (on the official letterhead) for the contents of the container and a package certificate; a photocopy of the strain passport with its complete characteristics; a special cargo transportation certificate with information about the consignee, date of dispatch and type of transport. The vial with the freeze-dried culture was wrapped with hygroscopic cotton, placed into the container, the first copies of the listed documents were put inside (Fig. 2A, B).

The container was hermetically sealed with a lid, wrapped in paper, laced and stamped with a wax seal bearing special signs "Caution! Do not open during transportation!" (Fig. 3A, B, C, D).

CONCLUSION

Analysis of the data obtained during the transfer of *B. mallei* strain 5584 from the Laboratory for Collection of Strains of Microorganisms in the FSBSI "FCTRBS-ARRVI" to FKP "Kursk Biofactory – BIOK Company" allowed us to assess the actions taken in compliance with Rules of SP 1.2.036-95 and SP 1.3._-17. Study of viability and biological properties of production strain 5584 showed that the Laboratory for Collection of Strains of Microorganisms of the Institution provides optimal conditions to preserve the strain viability and original biological properties after 5 years of storage.

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