

Name of the project: IRN AP19677302 "Development of an IHA-kit based on recombinant proteins for the diagnosis of brucellosis."

Relevance: The epizootic and epidemic situation of brucellosis in the Republic of Kazakhstan (RK) remains very complicated. More than 60% of rural districts are affected by animal brucellosis, which does not allow reducing the incidence of this disease among people. Early diagnosis of animal brucellosis is a key link in the overall system of veterinary and sanitary measures aimed at eradicating this infection. Traditional serological tests, including commercial diagnostic kits based on enzyme linked immunosorbent assay (ELISA), detect antibodies that are specific to smooth lipopolysaccharides (S-LPS) of the *Brucella* cell wall. The latter can cross-react with closely related bacteria due to the similarity of antigenic epitopes, leading to false positive results. For Kazakhstan, which ranks 9th in its territory and has an insufficient level of equipment in diagnostic laboratories, a diagnostic test is needed that is not inferior to ELISA/S-LPS in sensitivity, but surpasses it in specificity and is suitable for mass animal testing for brucellosis. Such a diagnostic test can be developed on the basis of an indirect hemagglutination assay (IHA) in the presence of an antigen specific for *Brucella*.

The aim of the project is to develop an IHA kit for the serological diagnosis of brucellosis based on the use of erythrocytes sensitized with *Brucella* spp. combined recombinant proteins expressed by prokaryotic producer strains.

Expected results

Upon completion of the project the following results will be achieved:

Normative and Technical Documentation: Standard of the organization, Instructions for the manufacture and control of the "IHA-kit based on recombinant proteins for the diagnosis of brucellosis" ("IHA-kit ...") and Instructions for the use of the "IHA-kit ..." will be developed;

- an experimental batch of "IHA-kit based on recombinant proteins for the diagnosis of brucellosis" will be manufactured;

- 2 (two) original papers in peer-reviewed scientific journals indexed in the Science Citation Index Expanded Web of Science database and (or) having a CiteScore percentile in the Scopus database of at least 35 (thirty-five) and a patent application for Derwent Innovations, as well as a paper in a peer-reviewed domestic journal recommended by the CQASHE, MSHE, RK, will be published;

- the results will be reported at 3 (three) scientific conferences, including international conference abroad with the publication of abstracts and/or papers in proceedings;

- seminars will be held for employees of veterinary and medical diagnostic laboratories, as well as practitioners on the use of the "IHA-kit ...";

- a web page will be created on the Applicant's website, which will contain information on the results of the project in order to search for partners interested in the commercialization of "IHA-kit ...";

- the thesis will be defended for the academic degree of Master of Science in the Educational Program "Veterinary Biotechnology"; the thesis will be completed for the academic degree of PhD in the Educational Program "Veterinary Medicine", and 6 (six) diploma works will be defended by bachelor students.

During the project implementation in 2023:

- bacterial suspensions of strains producing *Brucella* recombinant outer membrane (Omp19, Omp25, Omp31), periplasmic (BP26, SOD) and chimeric proteins (Omp19+25, Omp19+31, Omp25+31) were accumulated;

- purified preparations of these recombinant proteins were prepared;

- rabbit antisera against whole cells of *Brucella abortus* 19 and *B. melitensis* Rev1 were obtained;

- mouse antisera were obtained against whole cells of related bacteria, such as *Escherichia coli* O157:H7, *Campylobacter fetus*, *Salmonella enteritidis* и *Pasteurella multocida*;
- the antigenicity and specificity of *Brucella* outer membrane and periplasmic recombinant proteins were studied by indirect ELISA, immunoblotting and RID;
- the optimal conditions for the sensitization of erythrocytes by recombinant proteins were worked out and its specificity was studied in homologous and heterologous sera using IHA.

Members of the Research group:

Project leader - Bulashev Aitbay Kabykeshovich, Doctor of Veterinary Sciences, Professor of the Department of Microbiology and Biotechnology of KATRU, Scopus Author ID: <https://www.scopus.com/authid/detail.uri?authorId=7801312328>; <https://www.webofscience.com/wos/author/record/GEF-9526-2022>; Researcher ID: O-7397-2017, ORCID: <http://orcid.org/0000-0002-8427-509X>;

Research group:

- Eskendirova Saule Ziyadinovna, Candidate of Veterinary Sciences, Associate Professor, Leading Researcher of the Laboratory of Cellular Biotechnology of the National Center for Biotechnology (NCB), project position: Leading Researcher: Scopus Author ID: 55438123400; ORCID 0000-0002-9570-7433; Research ID O-2344-2017;

- Akibekov Orken Sultankhamitovich, Ph.D., Associate Professor of the Department of Microbiology and Biotechnology of KazATRU, project position: Senior Researcher, Scopus Author ID: 56606295400, Researcher ID: O-7690-2017, ORCID: <https://orcid.org/0000-0002-8647-0083>;

- Syzdykova Alfiya Safiollaevna, Master of Science, Researcher of ABRP KazATRU, project position: Researcher, Scopus Author ID: 57193998019; Researcher ID: AAE-7700-2022, ORCID: <https://orcid.org/0000-0002-5405-2469>;

- Ingirbai Bakytkali, PhD, NCB, Researcher, Laboratory of Genetic Engineering, project position: Researcher; Scopus Author ID: 57193571536, ORCID: 0000-0002-6915-8207;

- Zhumalin Aibek Khasietovich, Master of Agricultural Sciences, Leading Researcher of ABRP KazATRU, project position: Researcher, Scopus author ID: 57192061558, Researcher ID: AAE-7767-2022, ORCID: <https://orcid.org/0000-0002-8661-7348>;

- Zhagipar Fariza Sabitkyzy, Sampling and Information Processing Manager of the Kazakh-Chinese Laboratory for Biosafety KazATRU, project position: Researcher, Researcher ID: AAE-7613-2022, ORCID: <https://orcid.org/0000-0001-5296-1127>;

- Vacancy: junior researcher;

- Vacancy: laboratory assistant.

List of publications and patents published within the framework of this project: (with links to them)

Publications over the past 5 years:

1. Bulashev A.K., Jakubowski T., Mukantayev K.N., Tursunov K., Kiyan V., Zhumalin A. Using combined recombinant protein in the diagnosis of bovine brucellosis // *Medycyna Weterynaryjna*.- 2018.- Vol. 74 (3). – P.193-198; Q4/процентиль 28; цитирование 0/0; DOI: [dx.doi.org/10.21521/mw.6079](https://doi.org/10.21521/mw.6079);

2. Bulashev A.K., Jakubowski T., Tursunov K., Kiyan V., Zhumalin A. Immunogenicity and antigenicity of *Brucella* recombinant outer membrane proteins// *J. Veterinarija ir Zootechnika (Vet Med Zoot)*.- Vol. 76 (98).- 2018.-P.17-24; Q4/процентиль 16, цитирование 1/2; <https://vetzoo.lsmuni.lt/2018-76-en/>;

3. Kiyan V.S., Bulashev A.K., Katokhin A.V. *Opisthorchis felinus* and *Metorchis bilis* Metacercariae in Cyprinid Fish *Leuciscus idus* in Nura-Sarysu River, Kazakhstan // *Korean Journal of Parasitology*. - 2018. - Vol.56, No.3. - P.267-274; Q2/процентиль 38; цитирование 2/1.; <https://doi.org/10.3347/kjp.2018.56.3.267>.

4. Bulashev A.K., Akibekov O., Syzdykova A., Suranshiyev Zh., Ingirbay B. Use of recombinant Brucella outer membrane proteins 19, 25, and 31 for serodiagnosis of bovine brucellosis // *Veterinary World*. - 2020.-Vol. 13(7). - P.1439-1447; Q1, процентиль 79; цитирование 1/1 ; doi: www.doi.org/10.14202/vetworld.2020.1439-1447.
5. Kiyani V., Bulashev A., Zhumalin A., Smagulova A., Lider L. Immunogenicity and antigenicity of *Opisthorchis felinus* proteins // *Adv. Anim. Vet. Sci.*-2020.- Vol.8(9)-P. 933-939; Q3/ процентиль 39; цитирование 0/0; <http://dx.doi.org/10.17582/journal.aavs/2020/8.9.933.939>.
6. Ryskeldinova S., Zinina N., Kydyrbayev Z., Yespembetov B., Kozhamkulov Y., Inkarbekov D., Assanzhanova N., Mailybayeva A., Bugybayeva D., Sarmyikova M., Khairullin B., Tabynov K., Bulashev A., Aitzhanov B., Abeuov Kh., Sansyzybay A., Yespolov T., Renukaradhya G., Olsen S., Oñate A. and Tabynov K. Registered Influenza Viral Vector Based Brucella abortus Vaccine for Cattle in Kazakhstan: Age-Wise Safety and Efficacy Studies // *Frontiers in Cellular and Infection Microbiology* // published: 01 July 2021, Volume 11, Article 669196; Q1/процентиль 72; цитирование 0/0.; doi: 10.3389/fcimb.2021.669196.
7. Bulashev A., Suranshiyev Zh., Serikova Sh. Biotechnological methods in the diagnosis of brucellosis: scientific monograph. Nur-Sultan: Polygraphic Center of the Office of the President of the Republic of Kazakhstan. - 2021.- 295 p.[Kazakh].
8. Sotnikov D., Barshevskaya L., Zherdev A., Eskendirova S., Mukanov K., Ramankulov E., Dzantiev B. Immunochromatographic system for serodiagnostics of cattle brucellosis using gold nanoparticles and signal amplification with quantum dots // *Applied sciences*.- 2020.-V. 10.-P.738. IF 2,6 / Q3; doi:10.3390/app10030738.
9. Bulashev A.K., Ingirbay B.K., Syzdykova A.S., Kurmasheva A.K. Obtaining Brucella multiproteins and study of their immunoreactivity // *J. Biotechnology: Theory and Practice*.- 2021.-№ 1; doi.org/10.11134/btp.1.2021.6. [Kazakh];
10. Bulashev A.K., Ingirbay B.K., Mukantayev K.N., Syzdykova A.S. Evaluation of chimeric proteins for serological diagnosis of brucellosis in cattle // *Veterinary World*.-2021.- Vol.14(8). - P.2187-2196; Q1 процентиль 79; цитирование 0/0; doi: www.doi.org/10.14202/vetworld.2021.2187-2196;
11. Bulashev A.K., Akibekov O.S., Syzdykova A.S., Suranshiyev Zh.A. and Eskendirova S.Z. Serological potential of Brucella spp. recombinant proteins in the diagnosis of cattle brucellosis // *Bulletin of Science, Novosibirsk State Agrarian University*.-2020.-Vol.1(54).-P.56-64; doi: 10.31677 / 2072-6724-2020-54-1-56-64 [Russian];
12. Bulashev A.K. , Syzdykova A.S., Suranshiyev Zh.A., Tursunov K.A., Eskendirova S.Z. Objective assessment of the potential of recombinant proteins in the diagnosis of brucellosis // *Veterinary of farm animals (RSCI)*.-2020.-№1.-p.63-72; <https://elibrary.ru/item.asp?id=42631174> [Russian];
13. Barshevskaya L., Sotnikov D., Zherdev A., Khassenov B., Baltin K., Eskendirova S. , Mukanov K., and Dzantiev B . Triple immunochromatographic system for simultaneous serodiagnosis of bovine brucellosis, tuberculosis and leukemia // *Biosensors*.- 2019.-V. 9(4).-P.115; doi:10.3390/bios9040115. IF 5,5 / Q1;
14. Bulashev A.K., Akibekov O., Suranshiyev Zh., Ingirbay B. and Eskendirova S. Serodiagnostic potential of Brucella outer membrane and periplasmic proteins // *Turkish Journal of Veterinary and Animal Sciences*.-2019.-V. 43.-P. 486-493; Q3/процентиль 51; цитирование 1/2; doi:10.3906/vet-1902-75;
15. Sotnikov D., Berlina A., Zherdev A., Eskendirova S., Mukanov K., Ramankulov E., Dzantiev B. Immunochromatographic serodiagnosis of brucellosis in cattle using gold nanoparticles and quantum dots.// *International Journal of Veterinary Science*.- 2019.-V. 8(1).-P.28-34. IF 0,9 / Q4; [https:// www.researchgate.net/publication/332153577](https://www.researchgate.net/publication/332153577)
16. Mukantayev K., Tursunov K., Ingirbay B., Adish Z., Azhibayeva M. and Kairova Z., Ramankulov E., Mukanov K., Shustov A. Immunochromatographic assay for the foot-and-mouth disease utilizing recombinant nonstructural proteins 2C, 3A, 3B and 3D // *Bulgarian*

Journal of Agricultural Science.-2018.-Vol.24(3).-P.489-49; Q3;
<https://www.agrojournal.org/24/03-21.html>;

17. Bulashev A.K., Suranshiev Zh.A., Akibekov O.S., Akanova Zh.Zh. and Abulgazimova G. Serological diagnosis of cystic echinococcosis in cattle // *Folia Parasitologica* 2017, 64: 005 Q3/процентиль 46; цитирование 7/0; doi: 10.14411/fp.2017.005;

18. Bulashev A.K., Tursunov K.A. and Zhumalin A. Kh. The use of recombinant Brucella proteins for the detection of antibodies in seropositive animals // *Bulletin of Science, KATU.*- 2017.-Vol.92(1).-P.65-74;
<https://bulletinofscience.kazatu.edu.kz/index.php/bulletinofscience/article/view/419/379>;
[Russian].

19. Bulashev A.K., Tursunov K.T., Kairova Zh. K. and Syzdykova A. Obtaining Brucella abortus recombinant Omp19 and studying its antigenicity // *Bulletin of Science, KATU* - 2018.-Vol.98(3).-P.117-127, [Russian].
<https://bulletinofscience.kazatu.edu.kz/index.php/bulletinofscience/article/view/351/322>;

20. Bulashev A.K., Akibekov O.S., Suranshiev Zh.A., Syzdykova A.S., Ingirbay B.K. The use of protein antigens in the serodiagnosis of cattle brucellosis // *Bulletin of Science, KATU.*-2019.-Vol.101(2).-P.92-102;
<https://bulletinofscience.kazatu.edu.kz/index.php/bulletinofscience/article/view/257/234>,
[Kazakh];

22. Bulashev A.K., Akibekov O.S., Suranshiev Zh. A., Syzdykova A., Ingirbai B. Detection of antibodies to Brucella in the serum of vaccinated heifers // *Bulletin of Science, KATU.*-2020.-Vol.105(2). - P.181-189;
<https://bulletinofscience.kazatu.edu.kz/index.php/bulletinofscience/article/view/115/99>,
[Kazakh];

Information about Bulashev A.K. - Project Leader's patents for the last 5 years on the subject of the project;

- Patent No. 33124, Republic of Kazakhstan. IPC C12T 15/00 (2006.01). E. coli BL21 (DE3)/pET32/OmpBmBa - producer of recombinant chimeric protein of Brucella outer membrane protein / Bulashev A.K., Tursunov K.A., Zhumalin A.Kh., Mukantaev K.N.; applicant and patentee of JSC "Seifullin Kazakh Agrotechnical University". - 2017/0451.1; appl. 25.05.2017; publ. 24.09.2018, Bull. No. 36. - 6 p. [Russian];

- Patent No. 35776, Republic of Kazakhstan. IPC S12T 15/00. E. coli BL21(DE3)/pET28/Omp19/31 - producer of chimeric recombinant Brucella outer membrane protein/ Bulashev A.K., Akibekov O.S., Ingirbay B.K., Mukantaev K.N., Syzdykova A. S., Suranshiev Zh.A., Tursunov K.A.; applicant and patent holder JSC "Seifullin Kazakh Agrotechnical University". - 2021/0301.1; appl. 05/17/2021; publ. 07/29/2022, Bull. No. 30-8 p. [Russian].

Information for potential users:

The introduction of the "IHA-kit ...", made from domestic components, into diagnostic practice will significantly increase the effectiveness of veterinary measures due to the simplicity and low cost of the diagnostic tool, and recombinant proteins, first used in world veterinary practice as an erythrocyte antigen, will increase the specificity of serological testing, excluding false-positive results characteristic of classic serological tests.

"IHA-kit ..." can also be used in the diagnosis of human brucellosis, thus it will have a multiplier effect in the eradication of this socially dangerous zoonosis.

The diagnosis of brucellosis, as well as the diagnosis of other especially dangerous animal diseases, is classified as a state monopoly, therefore the main consumers of the "IHA-kit ..." will be the Republican Veterinary Laboratory, its regional and district divisions, as well as the National Reference Center for Veterinary Medicine, RK.

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