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ВЕСТНИК

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

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NAS RK is pleased to announce that Bulletin of NAS RK scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of Bulletin of NAS RK in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential multidiscipline content to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабаршысы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Web of Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабаршысының Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді мультидисциплинарлы контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Вестник НАН РК» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Вестника НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному мультидисциплинарному контенту для нашего сообщества.

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DEVELOPMENT OF METHODS FOR STUDIES ON BRUCELLOSIS IN THE MILK OF GOATS AND CAMELS

Abstract. Research of milk of goats and camels on brucellosis is carried out with the help of a diagnostic kit, which consists of the following components:

1. Color antigen 1 vial (ampoule) with a volume of 2.0 cm^3.
2. Positive brucella serum of animals, 1 vial (ampoule) in a volume of 2.0 cm^3.
3. Negative serum of animals, 1 vial (ampoule) in a volume of 2.0 cm^3.
4. Freeze-dried or milk healthy by cow brucellosis, 1 bottle of 20.0 cm^3.
5. distilled water in sterile form 1 vial (ampoule) in the amount of 20.0 cm^3 (for dilution of dried milk lyophilic).

On vials, ampoules of each biocomponent paste the label and pack 1 piece in cardboard boxes with the presence of nests or partitions, ensuring the immobility and integrity of the vial (ampoules).The diagnostic kit is stored indoors in a dark dry place at a temperature of 2 to 14 °C. The shelf life of the set is 12 months from the date of manufacture. In the presence of foreign matter, violation of tightness of bottles (ampoules), the absence of the label of the bottle (ampoule) is rejected and must be destroyed.

The procedure for applying the diagnostic kit. Florinsky test tubes are placed in the appropriate racks and numbered in accordance with the inventory of milk samples. In tubes pour 1.5 cm^3 of milk from the number of samples and add 0.5 cm^3 of cow milk, then add 0.05 cm^3 of antigen. All components of the reaction are thoroughly mixed and the tubes with the contents are placed in a water bath or thermostat at 37-38 °C for 60 minutes, until a blue ring appears in the control tubes.

At each formulation of the reaction, controls are set at the same time as the tested milk samples:

1. Goat milk (camel) with the addition of a positive serum in an amount of 0.05 per 1 cm^3 of milk;
2. Healthy goat (camel) milk with the addition of negative serum in the amount of 0.05 per 1 cm^3 of milk.

Accounting and evaluation of the results of the ring milk sample.

The results of the reaction are taken into account visually immediately after removing the racks from the water bath (thermostat) according to the following scheme (in crosses):

+++ (3 crosses) - a clearly defined blue ring in the upper part of the milk column in the cream layer (the rest of the milk remains white);

++ (2 crosses) - a fairly expressed blue ring in the cream layer (the rest of the milk has a bluish color);

+ (1 cross) - the blue ring in the cream layer is weakly expressed, and the whole column of milk is blue;

"- "(minus sign) - the column of milk remains uniformly colored in the original blue color, which was obtained immediately after the addition of antigen, and a layer of cream-white and slightly yellowish.

All milk samples that gave a ring reaction with a rating of 3 and 2 crosses are considered positive, and with a rating of one cross - doubtful.

Relevance. Diagnosis of brucellosis is one of the main links in the general complex of antiepizootic measures. Currently existing means and methods of combating brucellosis do not fully meet the requirements of practice [1].

Thus, the veterinary-sanitary rules for the control of brucellosis include blood serum (RBS, SBR/RLCF, ELISA), milk (CR and ELISA), biomaterial for bacteriological studies by isolating a pure culture, setting a bioassay, carrying out PCR.

However, the verification of animal products for food safety remains important. In this case, the milk of cows is investigated using a ring reaction [3]. The milk of some other species of animals (goats, camels) is not possible to subject to the study specified diagnostic test, due to the physico-chemical characteristics of the specified product [4].

The aim of our work was to develop a research method for brucellosis of goat and camel milk. To achieve this goal, the resolution has the following tasks:

1. To clarify the epizootic situation on the brucellosis of goats and camels in the Republic of Kazakhstan (determine relevance);

2. To study the possibility of researching milk obtained from goats and camels for brucellosis with the help of color antigen;

3. To give a comparative assessment of the methods of diagnosis of brucellosis in lactating goats and camels;

4. Develop a research methodology for brucellosis of goat and camel milk.

Research results.

Epizootic situation on camel brucellosis in the Republic of Kazakhstan. According to statistics, there are currently about 164,000 camels in Kazakhstan. At the same time, 199318 was subjected to research, taking into account repeated studies. The number of camelheads and the results of production studies for brucellosis are shown in table 1 and 2.

Table 1 – The number of camels in Kazakhstan by region

Name of regions	The number of brutes			
	all categories of farms	agricultural enterprises	peasant (farmer)	households (farmstead)
Akmola	140	112	20	8
Aktobe	14 867	210	7860	6797
Almaty	7 960	5205	2268	487
Atyrau	28 333	1936	10699	15698
West Kazakhstan	2 885	304	1708	873
Jambyl	5 530	153	3375	2002
Karagandy	1 207	4	603	600
Kostanay	177	26	82	69
Kyzylorda	34 471	1872	10253	22346
Mangystay	47 209	2622	16102	28485
South Kazakhstan	20 408	2941	5551	11916
Pavlodar	139	102	28	9
North Kazakhstan	56	3	53	–
East Kazakhstan	569	116	423	30
In total in RK	164 000	15606	59125	89320

As can be seen from table 1, the largest number of camels are concentrated in Mangistau, Kyzylorda, Atyrau, South Kazakhstan, Aktobe, then Almaty, Zhambyl, West Kazakhstan, Karaganda and other regions.The main population of camels is currently concentrated in private business entities, which focuses our attention on the epizootic well-being of camel head, especially in zooanthroponosis, in particular brucellosis. Milk and dairy products from these animals should be food safe. However, there are still no methods for the study of camel's milk for brucellosis and are not used in practical veterinary practice.

In connection with the above, official data on the presence of brucellosis infection among camel head have great interest.

The data of these studies, in the context of the areas presented in table 2.

Table 2 – Results of studies on camel brucellosis by region of Kazakhstan

Name of regions	It is investigational (heads)	Isolated patients	Incidence
Akmola	121	–	0,0
Aktobe	17 979	71	0,4
Almaty	9006	–	0,0
Atyrau	36 202	102	0,3
West Kazakhstan	3513	42	1,2
Jambyl	6544	2	0,03
Karagandy	1429	2	0,1
Kostanay	228	14	6,1
Kyzylorda	39 359	–	0,0
Mangystau	59 584	–	0,0
South Kazakhstan	24 574	–	0,0
Pavlodar	140	–	0,0
North Kazakhstan	40	–	0,0
East Kazakhstan	558	11	2,0
In total in RK	199 318	244	0,12

As can be seen from table 2, the number of positively responding to brucellosis, according to the veterinary reports, is in Kostanay (6.1%) East Kazakhstan (2.0), West Kazakhstan (1.2), Aktobe (0.4), Atyrau (0.3), Karaganda (0.1).

On average in Kazakhstan, the incidence of camel brucellosis in 2014 was 0.12%, in the Kostanay region this figure was 6.1% in East Kazakhstan - 2.0%, West Kazakhstan - 1.2%, Aktyubinsk - 0.4% Atyrau - 0.3%, Karaganda - 0.1%.

However, the data of veterinary reports do not always correspond to the actual position of the epizootic situation on camel brucellosis. Thus, for example, in the study of brucellosis, which is considered to be a successful camelhead in the village of Almaly-bak of the Almaty region, we found sick animals in the amount of 4 out of 22 studied, which is 18%.

Brucellosis infection among camelheads was not found in seven regions of the Republic, namely: Akmola, Almaty, Kyzylorda, Mangistau, South Kazakhstan, Pavlodar, North Kazakhstan.

However, the presence of brucellosis, even in a small number of camels, is a threat to the disease of a large number of people. The above data once again confirms the need for research on brucellosis of milk and dairy products, especially in regions with a significant spread of this infection.

As you can see, there is an urgent need for the timely detection of sick animals and their immediate isolation.

Data from veterinary laboratories show that camels are more likely to become infected with abortus brucella, that is, the infection comes from cattle.

However, in places of compact keeping the sheep head and camels together, which is often observed in human practice, there are risks of camels being infected from sheep by brucella of the type Melitenzis.

Epizootic situation on goat brucellosis in the Republic of Kazakhstan. According to the regional branches of the Committee for Veterinary Control and Supervision in the republic as a whole, there are some increased numbers of animals suffering from brucellosis.

In 2014, there were 114 disadvantaged points on brucellosis in the republic among small cattle, which contained 254,436 sheep and goats. In 2015, 53 new items were registered: in the Akmola region - 7; Aktobe - 5; East kazakhstan- 17; Zhambyl - 8; West Kazakhstan- 6; Karaganda - 9; Kyzylorda - 1.

In 2014, the highest incidence rates of sheep and goat brucellosis occurred in the Semipalatinsk region of the East Kazakhstan region - 1.24%, East Kazakhstan region - 0.83%, Zhambyl region - 0.70%, Almaty region - 0.56%, Taldykorgan region of Almaty region - 0 , 4%.

In 2015 (for the first quarter), fresh outbreaks of brucellosis were registered among small cattle –11, including 1 in the Akmola region; Atyrau - 2; east Kazakhstan - 1; Zhambyl - 4; West kazakhstan - 3.

The highest incidence rates of small cattle brucellosis in the named year were found in East-Kazakhstan region - 2.37%, Atyrau - 1.06%, and Almaty - 0.35%. Among the ill small cattle, there were goat brucellosis patients.

Thus, in particular, in the Almaty region in the period 2014-2015, according to the information of the regional veterinary laboratory, the following serological research data on brucellosis among goats were obtained (table 3).

Table 3 – Results of serological studies on brucellosis among goats

No.	Name of regions	2014 year		2015 year	
		number of samples	reacted positively	number of samples	reacted positively
1	Aksu District	26 873	234/0,87	46 062	263/0,57
2	Alakol District	11 755	58/0,49	21 509	73/0,33
3	Balkhash District	108	67/62	115	67/58
4	Enbekshykaz District	8700	153/1,75	4100	33/0,8
5	Eskeldy District	5751	–	12 951	2/0,01
6	Jambyl region	1521	11/0,72	527	–
7	Ile District	4542	110/2,42	785	33/4,2
8	Karasay District	265	6/2,26	119	11/9,2
9	Karatal District	6315	84/1,33	8424	300/3,56
10	Kerbulak District	12 903	94/0,72	19 859	51/0,25
11	Koksu District	29 045	–	6417	–
12	Panfilov District	338	–	6329	4/0,06
13	Raiymbek District	3040	36/1,18	1294	34/2,62
14	Sarkand District	11 568	99/0,85	14 460	215/1,48
15	Talgar District	2981	24/0,80	132	–
16	Uygur District	5231	60/11,47	529	27/5,1
17	Kapshagay	12 568	73/0,58	3944	43/1,09
18	Taldykorgan	3604	–	158	–
19	Tekeli	976	5/0,51	250	–
	Total	178 084	1114/0,62	147 964	1 157/0,78

Note. Reference designation: in fractional numbers the numerator – the absolute number; the denominator is the percent.

From table 3 it can be seen that in 2014, 178,084 goats were tested for brucellosis, in the first 6 months of 2015 - 147,964. Of the number of animals named in 2014, 1,114 had positive indications of immunological tests for brucellosis, and in 2015 - 1,157. As can be seen, the number of goats infected in the first 6 months of 2015 was higher than in the entire period of 2014.

However, fragmentary data available indicate the presence of brucellosis infection in goats. When studying the epizootic situation for goat brucellosis in the Almaty region, the presence of positively reacting animals in the listed prosperous business entities was noted.

The above data indicates the presence of brucella infection both among camelheads and goats in many territories of the Republic of Kazakhstan, which naturally requires an additional and careful approach to the assessment of the products obtained in terms of its food safety.

Exploring the possibility of researching milk obtained from goats and camels for brucellosis with the help of color antigen. In the literature (Shvartsman Y.S., Khazenson, LB, 1978) [5] there are reports on the development of so-called “local” immunity in animals, i.e. there is a development of protective mechanisms in tissues, where pathogenic microorganisms parasitize. When brucella dwells in the mammary gland, the production of antibodies detected in the secreted secretion is observed. Based on this PP. Trilenko (1956) [3] developed a ring reaction with cow's milk. The essence of this method is that by adding a colored antigen to a sick cow's milk, an immune complex is formed, which, on standing, rises with fat globules up the milk column and the cream layer becomes the color of the colored antigen.

However, the physico-chemical properties of milk, as indicated above, of other animals (camels, goats) do not allow this reaction to be carried out, which requires special studies.

For this purpose, milk was taken from healthy and sick with brucellosis animals (goats and camels), isolated according to indications of serological tests.

At the same time, in healthy animals, as with cows' milk samples, the milk column in test tubes had a bluish color, that is, the colored antigen was distributed evenly throughout the entire volume of the test material. In positive cases, the antigen-antibody complex settled to the bottom of the tube as an agglutinate. In the milk of healthy animals, the milk column remains uniformly colored blue (see figures 1, 2). This test is called a sedimentary reaction.

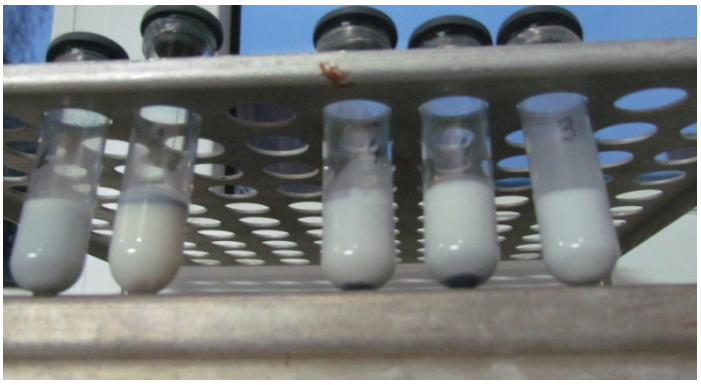

Figure 1 – Indications of the immunological reaction in the study of the brucellosis of the milk of goats and camels.
The two tubes on the left are the reaction with cow's milk (the left one is from a healthy animal; the right one is from a sick cow),
the next two tubes, to the right of those called goat and camel milk (without the addition of cow's milk),
the last right tube is the milk of a healthy animal.

Figure 2 – Sedimentary reaction with goat and camel milk

When taking milk samples for research, it is important to know that it must be fresh and delivered to the laboratory and examined on the day of the sampling. If this is not possible, the milk can be preserved with dry boric acid (0.1 g per 10 cm^3). Canned milk is suitable for research within 10 days.

At the same time, blood was drawn into vacutainers intended for obtaining serum.

A sedimentary reaction with samples of goat and camel milk was set up similarly to a ring reaction with cow's milk. The reading of the results of the sedimentary reaction was carried out according to the precipitate formed and the degree of staining of the milk column, as shown in the figure above. The obtained data of the sedimentary reaction were compared with the results of serological studies of blood

serum. The epizootological data on the welfare of herds of goats and camels for brucellosis was taken into account.

Lactating animals were unfavorable for brucellosis, according to the data of veterinary reporting and the results of our research.

The results of our studies of milk using the sedimentary reaction and blood serum samples of the abovementioned serological tests are reflected in the following table 4.

Table 4 – Comparative results of studies on serum brucellosis and camel whole milk

Number of animals	Indications of the sedimentary reaction		Results	
			AR	CBR
	with whole milk	divided 1:2	with blood serum	
1	#	#	1:400 +++	1:10 +++
2	#	#	1:400 +++	1:10 +++
3	#	–	1:100 +++	1:10 –
4	#	#	1:400 +++	1:10 #
5	+++	–	1:100 ++	1:10 –

Note. Reference designation: AR – agglutination reaction; CBR – complement binding reaction; SR – sedimentary reaction.

Comparing the data in table 4, we can state a definite correlation in the degree of immunological tests among themselves.

In the study of milk from animals No. 3 and 5, where negative results were obtained from studies of the secretion of the mammary gland at a dilution of 1: 2, there was a negative CBR result. As can be seen there are discrepancies in the results of studies of blood serum on various immunological reactions.

The above data clearly indicate the possibility of making a diagnosis of brucellosis by examining the milk of camels with a colored antigen intended for ring reaction.

The data obtained were the basis for the development of a research method for brucellosis of goat and camel milk using cow's milk.

Development of a research method for brucellosis of goat and camel milk based on a ring reaction. It was shown above that in the study of goat and camel milk, the appearance of a blue ring consisting of a colored antigen and antibodies, that is, an immune complex, is often not detected. At the same time, we noted that in milk of cows with the presence of brucella antibodies in it, when the color antigen is added, the immune complex rises with fat globules and a blue ring is formed in the upper part of the milk column. Considering these data, we attempted to study goat and camel milk, where fresh cow's milk was added as a solvent, in a 1: 1 ratio. From other components, positive brucella serum with a titer of 1:80 (+++), negative serum of goats and camels, commercial colored antigen, intended for ring reaction with cow's milk, were taken. The results of these studies are reflected in table 5.

From the data of table 5 it can be seen that the positive result of the ring reaction is marked with camel milk by adding to it positive brucella serum in an amount of 0.003125, which is its dilution of 1: 640. The results of these studies show that the ring reaction is more sensitive than the agglutination reaction.

Table 5 – Results of the ring reaction with camel's milk mixed 1: 1 with cow's

Reaction components	Amount of components, cm^3					
Camel milk Cow's milk	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0
In total	2,0	2,0	2,0	2,0	2,0	2,0
Positive Brucella serum	0,1 (1:20)	0,05 (1:40)	0,025 (1:80)	0,0125 (1:160)	0,00625 (1:320)	0,003125 (1:640)
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05
Results	#	#	#	#	+++	++
Control with negative serum	–	–	–	–	–	–

Thus, positive serum in RA had a titer of 1:80, and at the same time, in a ring reaction, the serum titer reached 320-640 units. The specificity of this reaction is shown by negative results with negative serum.

Similar results were obtained in the study of milk goats.

Next, we set the reaction with the same volume of positive serum, but with different dilutions. The results obtained are shown in table 6.

Table 6 – Data of the ring reaction with a mixture of milk of camels and cows with the addition of positive serum at different dilutions

Reactions components	Amount of components, cm^3					
Camel milk Cow's milk	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0
In total	2,0	2,0	2,0	2,0	2,0	2,0
Positive Brucella serum	0,2 (1:10)	0,2 (1:20)	0,2 (1:40)	0,2 (1:80)	0,2 (1:160)	0,2 (1:320)
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05
Results	#	#	#	#	#	+++
Control with negative serum	–	–	–	–	–	–

As can be seen from the data of table 6, the result was similar to the previous one.

The nature of the manifestation of the ring reaction with camel milk when diluted with cow's milk is shown in figure 3.

At the same time, in positive cases, a blue ring was formed, while without cow's milk a precipitate formed in this sample (figure 3).

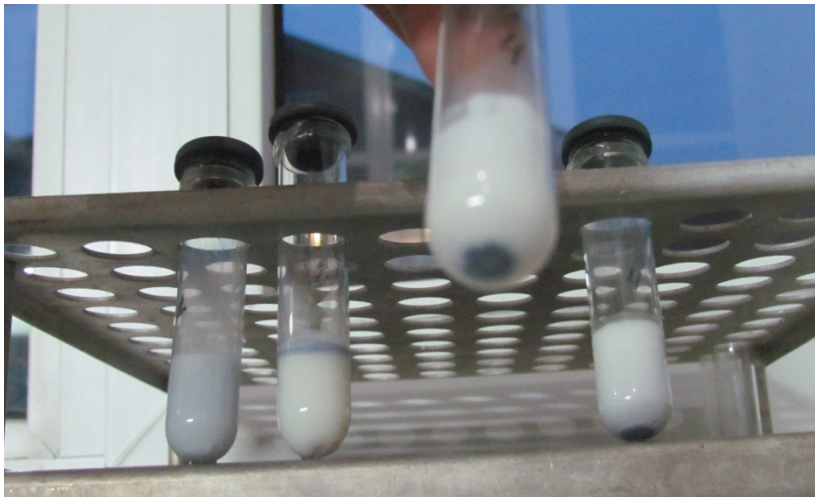

Figure 3 – The manifestation of positive and negative results of the reaction in the study of camel milk in its pure form and mixed with cow's

Note. From left to right: in the first test tube camel milk that does not contain brucella antibodies; in the second test tube camel milk with the addition of cow's milk and the presence of brucella antibodies; in the third and fourth tubes a positive sedimentation response.

As can be seen from figure 3, adding cow's milk to camel's milk with the presence of brucella antibodies in it causes, after 30-45 minutes aging at 37-38 °C, the appearance of a blue ring, which indicates a positive result of the ring reaction.

Next, we conducted studies to determine the amount of cow's milk required for the formulation of the ring reaction. For this purpose, we determined the ratio of cow's milk and camel's milk and carried out the formulation of the ring reaction.

The results obtained are reflected in table 7.

The data of table 7 show that the positive results of the ring reaction are clearly manifested when the content of cow's milk in camel in an amount of 20 percent or more.

Table 7 – The ratio of cow's milk and camel's milk in the ring dairy sample

Components	Quantity / cm^3					
Camel milk Cow's milk	1,0 1,0	1,2 0,8	1,4 0,6	1,6 0,4	1,8 0,2	2,0 –
The percentage of cow's milk in the total mixture	50	40	30	20	10	0
Total	2,0	2,0	2,0	2,0	2,0	2,0
Positive titer brucella serum 1:80 (+++)	0,25	0,25	0,25	0,25	0,25	0,25
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05
Results of RMT/SR	#/0	#/0	#/0	#/0	+++/+	0/#
Note. Reference designation: RMT – ring milk test; SR – sedimentary reaction.						

Similar results were obtained in the study of milk goats.

The above results indicate the possibility of using cow's milk as a dilution liquid.

Considering the mechanism of this reaction, we carried out studies of goat and camel milk using cow's milk of various fat contents as a diluent. The results obtained are reflected in table 8, 9.

Table 8 – Results of the ring reaction in the study of camel milk with a cow's milk content of various fat

Reactions components	Amount of components , cm^3					
Camel milk Cow's milk	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0
The percentage of fat cow's milk	4	2	1	0,5	0,25	0,125
Total	2,0	2,0	2,0	2,0	2,0	2,0
Positive titer brucella serum (1:80)	0,2	0,2	0,2	0,2	0,2	0,2
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05
Results	#	#	#	+++	++	+
Control with negative serum	–	–	–	–	–	–

Table 9 – Results of the ring reaction in the study of goat milk with the content of cow's milk of different fat content

Reaction component	Amount of components , cm^3					
Goat milk Cow's milk	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0	1,0 1,0
The percentage of fat cow's milk	3,6	2,5	1,5	1,0	0,5	0,25
Total	2,0	2,0	2,0	2,0	2,0	2,0
Positive Brucella serum (1:80)	0,2	0,2	0,2	0,2	0,2	0,2
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05
Results	#	#	#	+++	++	-
Control with negative serum	–	–	–	–	–	–

From the data in tables 8 and 9 it can be seen that the fat content in cow's milk for the manifestation of a pronounced ring reaction should be at least 1 percent.

The data we obtained made it possible to call this reaction an annular milk test (AMT) and use it in the study of milk, where the immune complex does not rise with fat globules, but precipitates in the form of agglutinate.AMT, in contrast to the ring reaction, has a complementary additive in the form of cow's milk (3 components), and in the ring reaction, unlike AMT, 2 components participate.

Thus, we have developed a new, previously unknown immunological reaction - an annular milk test, the procedure of which in generalized form is as follows.

In the tubes of Florinsky, goat or camel milk is poured in a volume of 1.5 cm^3. Then add 0.5 cm^3 of cow's milk with fat content of at least 1%. The mixture is thoroughly mixed and 1-2 drops of a colored

commercial antigen are added, after which the tubes with the contents are shaken and kept at 37-38 °C in a refrigerator or in a water bath for 30-45 minutes and the reaction is read. Evaluation of the results is carried out according to the following scheme and is conditionally expressed in crosses:

- the presence of a clearly defined ring in the upper part of the milk column, the rest remains white;

+++ - the presence of a fairly pronounced ring, the lower part of the milk has a slightly bluish color;

++ - the presence of a ring, the lower part of the milk has a blue color;

+ - weakly pronounced ring, and the milk column has a blue color;

– - The color ring is missing, the milk column remains evenly colored blue.

At the same time, controls are set with positive and negative milk samples.

Thus, as a result of our research, 2 methods have been developed to study the milk of goats and camels: a sedimentary reaction with the milk of lactating animals and an annular milk test, which can be used in the study of freshly given milk of goats and camels.

There are several varieties of the camel squad in the world: humpless (alpacas, llamas), single-humped (dromedary) and double-humped (bactrians). In the Republic of Kazakhstan, only single-humped and two-humped camels are engaged in breeding. In this regard, of great scientific and practical interest is the suitability of the methods developed by us for the study of milk from the above varieties of lactating animals.

Milk for research was obtained by placing it into sterile cones of four udder lobes. The study is subjected to a secret of the breast, taken from 22 lactating camels, of which 12 two-humped and 10 one-humped. Reactions were set by the methods described above. The results of these studies are reflected in the following table 10.

Table 10 – Indications of PR and RMT in the study of the brucellosis of milk obtained from dromedars and Bactrians

No	The number of animals and their epidemiological characteristics	Types of animals	Ring milk test (RMT)			Sedimentary reaction (SR)		
			positively	doubtful	negatively	positively	doubtful	negatively
1	12 dysfunctional	baktrians	5/41,7	2/16,7	5/41,7	5/41,7	2/16,7	5/41,7
2	5 successful	baktrians	–	–	5/100,0	–	–	5/100,0
3	10 dysfunctional	dromedary	4/40,0	1/10,0	5/50,0	4/40,0	1/10,0	5/50,0
4	5 successful	dromedary	–	–	5/100,0	–	–	5/100,0

From the data of table 10 it appears that the results of CMP completely coincide with the sedimentary reaction. The negative results of the study of safe livestock indicate the specificity of the tests developed by us.

Thus, our proposed methodology for the study of camel's milk for brucellosis can be applied in the study of both Bactrians and dromedars, which increases their practical significance.

In connection with the above, comparative data from the study of milk and serum of the same animals are of interest. The data obtained are shown in table 11.

Table 11 – Results of studies of milk of camels with different epizootic characteristics of brucellosis and serum

No	The number of animals epizootological characteristic	RMT			RBS+AR+CFT (matches)		
		positively	doubtful	negatively	positively	doubtful	negatively
1	22 dysfunctional	9	3	10	4	–	18
2	10 successful	–	–	10	–	–	10
Note. Reference designation: RMT – Ring milk test; RBS – rose bengal sample; AR – agglutination reaction; CFT – complement fixation test.							

The data in table 11 show that the number of positive indications of the RMT is significantly greater than the number of positive results in the study of blood serum. It is important to note that all animals that reacted positively by serological tests gave positive indications in the study of milk by the ring dairy sample.

Similar results were obtained in the study of milk taken from 180 goats. The resulting data is shown in table 12.

Table 12 – Results of studies of milk and serum taken from goats in Almaty region

Amount of investigated animals	Blood test results						The results of the study of the secret of the breast			
	RBS		AR		CFT		OC		RMT	
	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.	pos.	neg.
Karasai district, village Algabas										
2	–	2	–	2	–	2	–	2	–	2
Karasai district, the village of Kemertogan										
5	–	5	–	5	–	5	–	5	–	5
Ilijskij district, village Burundaj										
54	–	54	–	54	–	54	–	54	–	54
Ili district, Eskeldi village										
7	7	–	7	–	7	–	7	–	7	–
Talgarsky district, Belbulak village (otgon)										
6	–	6	–	6	–	6	–	6	–	6
Talgar district, Panfilov village										
6	–	6	–	6	–	6	–	6	–	6
Talgar district, Kerbulak distant section										
28	–	28	–	28	–	28	–	28	–	28
Enbekshikazakh District, Karazhotin rural district										
72	–	72	–		72		–		–	72
Total amount	7	173	7	173	7	173	7	173	7	173

As can be seen from the data of table 12, the study of 180 animals revealed 7 positively responding to all diagnostic tests in the Ili district (p. Eskeldi -7).

These data indicate that in the mammary gland can develop anti-brutselleznyh protective substances, captured ring annular milk sample. These data are consistent with the available reports of special literature (Schwartzman, YS, Hazenson, L. B., 1978).

In connection with the results obtained by us, it is of great scientific and practical interest to study the presence of brucella antibodies in each of the 4 udder lobes. Milk for the study was obtained in sterile tubes separately from each udder portion.

The obtained data were compared with the results of serological studies of blood serum, which is reflected in table 13.

From the data in table 13, it can be seen that the reaction with milk from each udder lobe can have different results.

So, for example, from the first animal, the milk extracted from the left anterior lobe had a negative immunological test result. In the study of biomaterial taken from the second animal, in all cases a positive result was obtained. In the third animal, positive results were obtained in the study of milk only from the posterior shares of the udder. In the fourth animal, a negative result of the study was obtained with milk from the left anterior lobe of the udder.

Comparing these data with the results of serological studies, we can note a certain correlation between the results of the study of milk and serum.

Thus, in the study of milk from animal No. 3, where negative results of studies of secretion from the front parts of the udder were obtained, there was a negative result of the complement fixation reaction.

In addition, we noted a discrepancy between the results of studies of blood serum on various immunological reactions. The CSC readings in animal No. 3 were negative with positive results for RA, sedimentary reaction and ring milk test.

Table 13 – Comparative results of studies on serum brucellosis and camel whole milk

Number of animals	Udder shares from which milk was obtained for research	Milk Reaction Indications	Results	
			AR	CFT
1	1	#	1:400 +++	1:10+++
	2	–		
	3	#		
	4	#		
	5 (combined with all shares)	#		
2	1	#	1:400 #	1:10#
	2	#		
	3	#		
	4	#		
	5 (combined with all shares)	#		
3	1	–	1:100 +++	1:10 –
	2	–		
	3	#		
	4	#		
	5 (combined with all shares)	+++		
4	1	#	1:400 +++	1:10+++
	2	–		
	3	#		
	4	#		
	5 (combined with all shares)	#		
Note. Reference designation: Numbers indicate udder shares: 1 – front left; 2 – front right; 3 – rear left; 4 – back right.				

The above data clearly shows that when making a diagnosis of brucellosis, it is necessary to conduct comprehensive diagnostic studies of blood serum and milk. In addition, when examining milk it is necessary to take samples for research from each part of the udder.

Comparative evaluation of methods for diagnosing brucellosis in lactating camels. The diagnosis of animal brucellosis is carried out on the basis of data from the epizootology, clinical picture, pathoanatomical changes and the results of allergic and laboratory research.

In the laboratories most often carry out serological and bacteriological studies. At the same time, agglutination reactions, binding reactions (long-term) complement are used.

The facts, when at certain periods of the disease some reactions may be negative and others positive, confirm the need for a complex use of various immunological reactions, which greatly complements the possibility of more complete identification of animals with brucellosis.

Subsequent studies of milk taken from positively reacting camels showed a certain correlation in the severity of the results of studies of this product and blood serum.

The results of our research on the blood serum and milk of camels are shown in table 14.

Table 14 – Comparative results of studies of serum and milk of camels

No	Number of animals	Epizootic characteristics	The number of positive testimony			
			RMT	RBS	AR(1:200 and higher)	CFT
1	22	dysfunctional	4	4	3	3
2	10	successful	–	–	–	–
Note. Reference designation: RMT – Ring milk test; RBS – rose bengal sample; AR – agglutination reaction; CFT– complement fixation test.						

From table 14 it appears that all used serological tests for the diagnosis of brucellosis in camels are specific, as evidenced by the negative results of studies of a prosperous group of animals. At the same time, out of the number of unfavorable livestock, 4 animals (18.1%) had positive indications in the study of milk RMT and blood serum according to RBS and 3 animals (13.6%) reacted positively in AR and CFT.

Numerous studies of goat milk samples showed similar results.

Thus, our data confirm the need for comprehensive studies in the control of brucellosis in goats and camels.

Next, we carried out a positive treatment of serum in the agglutination reaction on a solution with a high content of sodium chloride (10%) and in the ring dairy sample.

The scheme of the stated reactions and the results of research are reflected in the following table 15.

Table 15 – Schemes of formulation of the RMT and AR and the results of titration of positive Brucella serum in the indicated reactions

Components	Number of test tubes								
Staging scheme of RMT									
Camel milk	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5	1,5
Cow's milk	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5	0,5
In total	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Positive Brucella serum	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
Color antigen	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Results of RMT	#	#	#	#	#	#	#	+++	++
Staging scheme of AR									
Positive Brucella serum	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2	0,2
10% sodium chloride solution	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0	2,0
Single Brucella antigen	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Results of AR	#	#	#	#	#	+++	+	–	–
Note. Reference designation: AR – agglutination reaction ; RMT – Ring milk test.									

From the data given in table 15 it is seen that the ring milk sample is more sensitive to the tube agglutination test not only when it is set to a 0.85% solution of sodium chloride, but also to a 10% solution of the specified salt.

Bacteriological studies of the milk of positively reacting goats and camels for the presence of brucella in it.

When studying the specificity of indications of a ring milk test and a sedimentary reaction, it is important to know not only the coincidence of the results obtained with the data of serological studies, but also the results of bacteriological findings.

For this purpose, milk was examined in parallel, taken from each udder lobe, ring serum sample and sedimentary reaction and subjected to bacteriological examination.

Milk from lactating animals was taken either by a catheter, or by issuing from each lobe of the udder to a separate tube. At the same time, camel milk visually did not have any pronounced differences from the milk of these animals, described in the special literature.

Sowing from bacteriological material was carried out on meat-peptone hepatic glucose-glycerol agar, meat-peptone glucose-glycerol broth. Crops were incubated in desiccators with a high content of carbon dioxide for 30 days with periodic viewing after 5-7 days. Colonies with suspicion of brucella were subjected to further study, in particular, carried out the agglutination with specific positive serum, viewed under a microscope smears, gram-stained. At the same time, milk samples in a volume of 1.5 cm^3 were injected subcutaneously to guinea pigs (bioassay), which were examined by serological tests. Upon receipt

of a positive result, it was believed that the presence of Brucella in milk occurred, even with a negative result of direct seeding. Laboratory animals with positive readings of the bioassay were killed and bacteriological sowings were carried out on nutrient media from parenchymal organs and lymph nodes.

Then the selected cultures were studied according to the differential table, the type and individual biovars were determined. According to the data obtained, they made a conclusion and developed appropriate measures.

Bacteriological examination of milk subjected to milk from four positive serological camels and seven goats. In this case, bacteriological seeding of breast secretion was carried out from each udder lobe. The obtained data were compared with the indications of serological reactions. The results of these studies are reflected in table 16.

Table 16 – The results of bacteriological studies of milk samples
with positive indications of the sedimentary reaction and the ring milk test

Number of animals	Indicates of SO and RMT	Results	
		Bacteriological research	biotests
Camel smilk			
1	Milk from the udder with a positive reaction, positive.	Brucella culture is highlighted	Positive
	Milk from udder fractions with a negative. reaction, negative.	Culture not highlighted	Negatiove
2	Milk from all parts of the udder with a positive and negative. Reactions positive.	Culture highlighted	Positive
3	Milk from udder with positive reaction, positive.	Culture highlighted	Positive
	Milk from the udder with the negative reaction, negative.	Culture not highlighted	Negative
4	Milk from udder with positive reaction, positive.	Culture not highlighted	Positive
	Milk from the udder with the negative reaction, negative.	Culture not highlighted	Negative
Goat's milk			
1	milk from the left udder, positive.	Culture not highlighted	Positive
	milk from the right udder, positive		
2	milk from the left udder, positive	Culture not highlighted	Positive
	milk from the right udder, positive		
3	milk from the left udder, positive	Culture not highlighted	Positive
	milk from the right udder, positive		
4	milk from the left udder, positive	Culture not highlighted	Positive
	milk from the right udder, positive		
5	milk from the left udder, positive	Culture not highlighted	Positive
	milk from the right udder, positive		
6	milk from the left udder, positive	Culture not highlighted	Positive
	milk from the right udder, positive		
7	milk from the left udder, positive	Culture highlighted	Positive
	milk from the right udder, positive		

Note. Reference designation: SR – sedimentary reaction; RMT – Ring milk test.

From the data in table 16 it can be seen that the positive results of the bioassay and the culture of brucella are distinguished mainly from those parts of the udder that had positive indications of the sedimentary reaction and the ring dairy sample. It is important to note that with a negative result of direct seeding, in some cases there may be a positive bioassay, which is important in practical terms when testing for brucellosis of biological material.

In this regard, the collected milk, which gave positive testimony from the SR and RMT, must be re-examined with samples taken from each udder fraction and when a positive result is obtained with this

sample, it is necessary to perform a biological test in order to isolate the culture and its subsequent identification and differentiation.

The results of these studies are of great scientific and theoretical and important practical importance.

Biological properties of Brucella cultures isolated from goats and camels. It is known that brucellae belonging to the species B.abortus are more often distinguished from patients with brucellosis of camels. However, camels, without having a typical pathogen of this disease, can become infected with brucella of the species B. melitensis when they are kept together with a livestock of small ruminants that are unfavorable for brucellosis. At the same time, anti-brunch activities are held somewhat differently.

In this connection, it is of interest to study the biological properties of brucella cultures isolated from camels. In the process of work, we isolated 5 cultures from 4 animals. At the same time, two cultures are isolated from bioassic guinea pigs.

The results of the differentiation of grown cultures are shown in table 17.

Table 17 – These differentiations of cultures of brucella isolated from camels

No culture	Necessity of CO_2	Allotment of H_2S	Growth on envirement containing				Agglutinin		Phagesensitivity
			thionin		magenta		A	M	
			1:50 thousand	1:100 thousand	1:50 thousand	1:100 thousand			
1	±	+	–	–	+	±	+	–	+
2	±	+	–	–	+	±	+	–	+
3	±	+	–	–	+	±	+	–	+
4	±	+	–	–	+	±	+	–	+
5	±	+	–	–	+	±	+	–	+
B.abortus 544	–	+	–	–	–	–	+	–	+
B.suis 1330	–	+	+	+	–	–	+	+	+
B.melitensis 567	–	–	+	+	+	+	–	+	–

The data in table 17 show that all isolated cultures of Brucella are of the species B.abortus 4th biotype.

Our data on the study of the biological properties of Brucella suggests that this camel population has been in contact with a dysfunctional herd of cattle.

The above shows that measures to combat brucellosis should be carried out similarly to the measures provided for the rehabilitation of cattle from brucellosis infection.

The results of the study of cultures of Brucella isolated from goats showed that they have typical morphological, tinctorial and biochemical (see figures 4, 5, table 18) properties characteristic of B. melitensis.

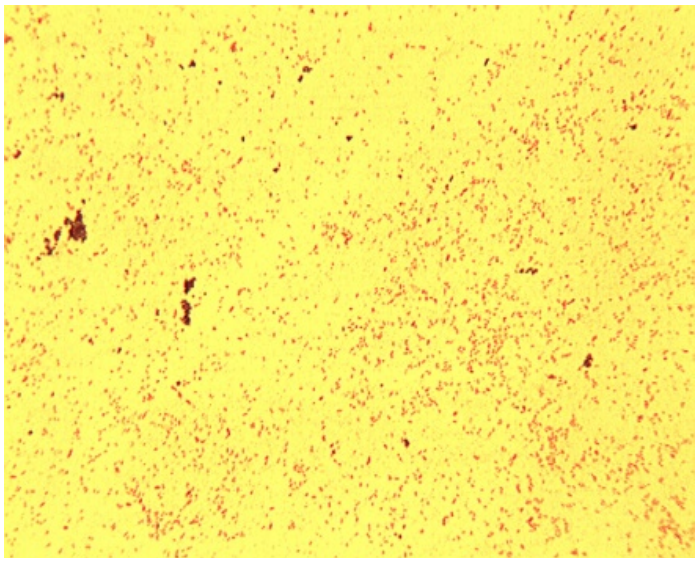

Figure 4 – Brucella melitensis

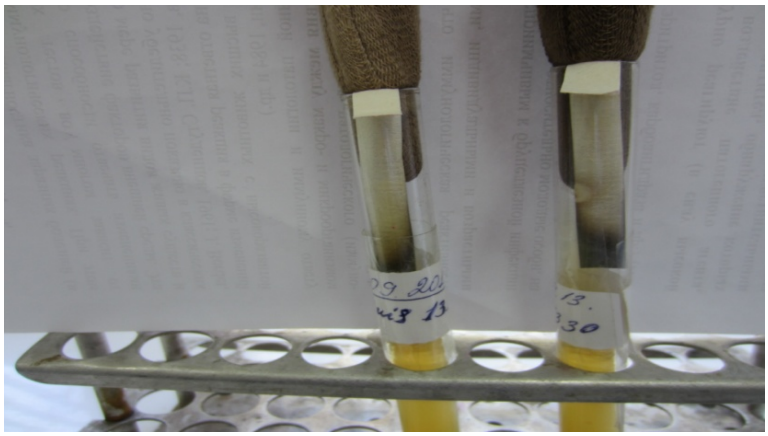

Figure 5 – Darkening of the strips of filter paper

Table 18 – These differentiations of cultures of brucella isolated from goats

No culture	Necessity of CO_2	Allotment of H_2S	Growth on envirement containing			
			thionin		magenta	
			1:50 thousand	1:100 thousand	1:50 thousand	1:100 thousand
1	–	± (3)	+	+	+	+
2	–	± (5)	+	+	+	+
B.abortus. 544	–	+ (7)	–	–	–	–
B.suis 1330	–	+ (13)	+	+	–	–

The data of table 18 show that all selected cultures of Brucella according to the applicable tests belong to the species B.melitensis.

Thus, according to the results of the research, we selected the selected cultures of Brucella to the species B. melitensis.

Given that the culture of the species Melitenzis is distinguished from goats, we have prepared a colored antigen for the study of goat milk from B. melitensis Rev-1.

In a comparative assessment of the effectiveness of commercial diagnosticum for the study of goat's milk (from Brucella abortus 19) and prepared by different methods from Brucella abortus 19 and Rev-1 merups, 18 samples of milk from goats were tested by ring dairy. The results obtained with this are summarized in table 19.

Table 19 – The results of the study of milk goats with colored antigens

Number of samples		Results of RMT with antigen									
		Commercial of brucella strain 19		prototypes of strains							
				19				Rev-1			
				AIEVM		M.J.Corbel (1992)		AIEVM		M.J.Corbel (1992)	
		pos	neg	pos	neg	pos	neg	pos	neg	pos	neg
18	abs	15	3	15	3	15	3	18	–	18	–
	%	83	17	83	17	83	17	100	–	100	–
Control	abs	–	3	–	3	–	3	–	3	–	3
	%	–	100	–	100	–	100	–	100	–	100

From the data presented in table 19, it is clear that prototypes of antigen from strain B. abortus 19 in all cases were not inferior to a commercial preparation. At the same time, diagnostic tests made from the B. melitensis Rev-1 strain confirmed all cases and additionally three samples were detected, which accounted for 17% of the indicated number of the studied material.

Thus, we have proposed a method of setting the reaction for the study of the milk of goats and camels for brucellosis. At the same time, it is more expedient to investigate milk with a colored antigen prepared from B. melitensis Rev-1+ B.abortus 19, which has homology with an antigenic structure with that of Brucella cultures circulating in an epizootic focus.

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ЕШКІ ЖӘНЕ ТҮЙЕ СҮТІН БРУЦЕЛЛЕЗГЕ ЗЕРТТЕУ ӘДІСТЕРІН ӘЗІРЛЕУ

Аннотация. Ешкі және түйе сүтін бруцеллезге зерттеу, диагностикалық жиынтық көмегімен жүзеге асырады, олкелесі компоненттерден тұрады:

1. Түсті антиген 1 флакон (ампула) 2,0 см^3 көлемде.
2. Бруцеллезге оң қан сарысуы, 1 флакон (ампула) 2,0 см^3 көлемде.
3. Бруцеллезге теріс қан сарысуы, 1 флакон (ампула) 2,0 см^3 көлемде.
4. Бруцеллезден сау сиырдың лиофильді кептірілген немесе тұтас сүті, 1 флакон, 20,0 см^3 көлемде.
5. Стерильді дистилденген су 1 флакон (ампула) 20,0 см^3 көлемде, (лиофилді кептірілген сүтті езу үшін).

Әрбір биокомпонеттің флакондарына, ампулаларына этикетка желімдейді және флаконның (ампуланың) қозғалмауы мен тұтастығын қамтамасыз ететін ұяшықтары немесе қалқалары бар картон қораптарға 1 данадан салынады. Диагностикалық жиынтықты қараңғы құрғақ жерде 2 °С-тан 14 °С-қа дейінгі температурада сақталады. Жиынтықтың жарамдылық мерзімі-дайындалған күннен бастап 12 ай. Флакондардың, (ампулалардың) герметикалығы бұзылса, бөгде қоспа болған жағдайда, флакондардың (ампуланың) этикеткасы болмаған кезде жарамсыз болады және жойылуға жатады.

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РАЗРАБОТКА МЕТОДОВ ИССЛЕДОВАНИЯ НА БРУЦЕЛЛЕЗМОЛОКА КОЗ И ВЕРБЛЮДИЦ

Аннотация. Исследования молока коз и верблюдицна бруцеллез осуществляет с помощью диагностического набора, который состоит из следующих компонентов:

1. цветной антиген 1 флакон (ампула) объемом 2,0 см^3;
2. позитивная бруцеллезная сыворотка крови животных, 1 флакон (ампула) в объеме 2,0 см^3;
3. негативная сыворотка крови животных, 1 флакон (ампула) в объеме 2,0 см^3;
4. лиофильно высушенное или цельное молоко здоровой по бруцеллезу коровы, 1 флакон объемом 20,0 см^3;
5. дистиллированная вода в стерильном виде 1 флакон (ампула) в объеме 20,0 см^3, (для разведения лиофильно высушеного молока).

На флаконы, ампулы каждого биокомпонента наклеивают этикетку и упаковывают по 1 штуки в картонные коробки с наличием гнезд или перегородок, обеспечивающих неподвижность и целостность флакона (ампул). Диагностический набор хранят в закрытых помещениях в темном сухом месте при температуре от 2 до 14 °С. Срок годности набора – 12 месяцев со дня изготовления. При наличии посторонней примеси, нарушении герметичности флаконов (ампул), отсутствии этикетки флакона (ампулы) бракуется и подлежит уничтожению.

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