European Journal of Agricultural and Rural Education (EJARE)
Available Online at: https://www.scholarzest.com
Vol. 2 No. 6, June 2021,
ISSN: 2660-5643

DETERMINATION OF IGG ANTIBODY AGAINST LEUKEMIA VIRUS IN CATTLE USING IMMUNOENZYME ASSAY

Razzaqova Dilorom Shuxratovna
Basic Doctoral Student (PhD) of the Veterinary Research Institute.
razzakova90@mail.ru

Article history:

Received April 26th 2021
Accepted: May 19th 2021
Published: June 15th 2021

Abstract:

Cattle leukemia is a chronic infectious disease that results from the poisoning of blood cells by the virus, leading to disruption of the genetic apparatus and poor cell quality. The proposed IFA method allows the detection of specific antibodies in infected animals from 6 months of age and older (calves fed oral milk and milk from infected cows can receive maternal antibodies stored for up to 6 months), so false positive results when serologically tested young calves can be obtained. It is also used in rapid screening to detect the presence of infected animals in the herd (up to 10 mixed serums are allowed in groups), and is used to confirm PTsR results when provirus DNA levels are minimal.

Keywords: Leukemia, sandwich method, nepryamoy method, IFA, antigen, specificity, DNA, genetic apparatus, serological method, milk, infectious disease, test express, PTsR

INTRODUCTION

The purpose of setting up an enzyme-linked immunosorbent assay is to detect IgG antibodies against the leukemia virus in cattle. The advantage of this test is that antibodies can also be detected in milk samples taken from cows. The result is evaluated by comparing the threshold coefficient = 1.0. If the optical density value is greater than the threshold coefficient, the samples are considered positive (i.e., contain antibodies against the leukemia virus).

The importance of enzyme-linked immunosorbent assay is that the test can be used to confirm results obtained by express methods, as well as to verify suspicious results obtained by other methods not based on the determination of antibody activity (e.g. PTsR).

Enzyme-linked immunosorbent assay (ELISA) allows the detection of specific antibodies in infected animals from 6 months of age and older (calves fed with colostrum and milk from infected cows can retain antibodies passed from their mother for up to 6 months.

METHODS USED IN THE STUDY.

During the study, the following methods were used for immunoenzyme analysis used in the detection of IgG antibodies against leukemia virus in cattle [1-6]:

1. Indirect enzyme-linked immunosorbent assay. In this case, a chain of complexes is formed by the antibodies present in the serum or cow's milk with the antigen immobilized on the surface of the polyethylene holes. The interaction of IgG monoclonal antibodies with the antivid conjugate of IgG in cattle with a special complex peroxidase changes the substrate and chromogen, more precisely, the color of the solution. The color intensity is directly proportional to the concentration of specific antibodies in the samples being tested.
2. Sandwich method. The sandwich is one of the heterogeneous IFA variants of the indirect "direct" method, which acts as an immunosorbent antibody.
3. Method of detection of "HEMA Leukemia IgG-IFA" in the test complex. Detection of IgG antibodies against the causative agent of bovine leukemia is based on the analysis of the "direct" method. The antigen is immobilized on the inner surface of the plate holes. The antibodies in the sample bind to the antigen on the surface of the hole. If the resulting complex is detected by pepper peroxidase using IgG antibodies of cattle, conjugate of mouse monoclonal antibodies, a "sandwich" containing peroxidase is formed.

The results of the study.

The antigen-retaining solution is added to the holes in which the pathogen antibodies are immobilized. In the first stage, the antigen-antibody complex is formed during the incubation process. The unbound components are washed and a special enzyme antibody is added. After the second incubation process and the elimination of the excess enzymatic conjugate, the enzymatic activity of the carrier, which is proportional to the initial concentration of the antigen under test, is determined. The compounds that make up an undyed sandwich are dyed by an enzymatic reaction (color reaction) under the influence of hydrogen peroxide and a substrate. Of course, the degree of color intensity depends on the amount of specific anteloles detected.

During incubation with tetramethylbenzidine (TMB) substrate solution, the solutions are stained in the form of holes. In this case, the color intensity is expressed in direct proportion to the antigen, the content of specific IgG antibodies.

A set of complexes was used to detect IgG antibodies against the causative agent of bovine leukemia in bovine serum, cow's milk, and pasteurized milk**.**

"HEMA Leukemia IgG-IFA "complex analysis revealed the following cases:

№	Code	naming	quantity	Unit of measurement	Color
1	P107B	Polystyrene tablet, divided into holes	1	piece	-
2	CN107BZ CP107BZ	When the control solutions (positive and negative) are ready for use, 1.5 ml.	2	piece	red
3	T107BZ	Conjugate, ready to use, 14 µl.	1	piece	red
4	S011Z	Buffer for dissolving samples, 50 ml.	1	piece	blue
5	S008Z	Concentrate of the solution for washing (saline solution Twin – 20 and mixed with benzoic acid, 26 times), 22 ml.	1	piece	No color
6	R055Z	Substrate solution - 3.3⁄, 5.5⁄- tetramethylbenzidine (TMB), 14 ml.	1	piece	No color
7	R050Z	Stop – reagent, 14 ml	1	piece	No color
8	N003	Film to cover the top of the tablets.	2	piece	-
9	K107BIR	Handbook "HEMA leukemia IgG – IFA"	1	piece	-

Important notes on reagent storage and testing:

1. Different series of reagents are not mixed or used in the same order.
2. The set shall consist of a set of 92 test specimens and 2 control sera for analysis in monoplicates.
3. For analysis, hemolyzed, turbid blood serum (plasma) as well as serum (plasma) containing sodium azide should not be used. Milk should be stored at a temperature of -20 ° C. The samples obtained cannot be re-frozen and thawed.
4. Close the cap immediately after using the reagent, especially if each vial is closed with its own cap.
5. All parts of the kit should be stored in the refrigerator (+2 ... + 8 ° C). Storage (transportation) of the package is allowed at a temperature of up to +25 ° C, not more than 15 days. It is not allowed to put the whole package in the freezer.
6. When used as a kit, components should be stored as follows:

- the remaining unused hole strips should be well sealed with paper to cover the plate and stored at a temperature of +2 ... + 8 ° C for the entire shelf life of the kit;
- conjugate, substrate, stop – reagent is stored at a temperature of +2 ... + 8 ° C for the entire shelf life of the package after opening the vials;

–control serum should be stored at a temperature of +2 ... + 8 ° C for no more than 2 months after opening;

For unused washing, the concentrate solution should be stored at a temperature of +2 ... + 8 ° C for the entire shelf life of the kit. The prepared washing solution should be stored at a temperature (+18 ... + 250C) for no more than 15 days or at a temperature of +2 ... + 8 ° C for no more than 45 days;

7. Do not use sodium azide preservatives to wash the microplate. Sodium azide, even in very small amounts, inhibits the marker enzyme peroxidase, which can lead to a weakened (passive) signal.
8. During all incubation processes, the tablet should be covered with a special film. It prevents the microplate holes from drying out during the process stages.
9. Cleaning of microplates can be carried out manually or on automatic equipment. 250 ml in each wash in the wells. a wash buffer of not less than No need to soak in the wash. After washing the microplates by hand, the buffer residue should be well wrapped in filter paper.
10. Using the substrate, the optical density is measured for 15 minutes after the reaction has stopped.
11. Strict adherence to the instructions for use of the kits is required to obtain a reliable result.

The special equipment used to obtain the results of scientific research consisted of the following components [7-9]:

- 450 nm vertical scanning photometer for measuring the optical density of samples in tablet holes;
- Thermostat that maintains a temperature of + 370S ± 20S;
- Interchangeable triplets designed for volume selection in the range of 10 to 250 µl;
- 1000 ml measuring cylinder;
- distilled water;
- rubber gloves;
- special filter paper.

PREPARATION OF AGGREGATE REAGENTS FOR THE ANALYSIS PROCESS:

1. Reagents and test specimens (blood plasma) in the package are stored at room temperature (+18 ... + 250C) for at least 30 minutes before the analysis.

2. Prepare the tablet. The tablet storage package is opened and the required number of strips are mounted on the tablet frame. In order to protect the unused strips from the effects of moisture, the tablet is glued with paper designed for gluing and stored at a temperature of +2 ... + 80C, during the storage period of the package [10-12].

PREPARE THE SOLUTION FOR WASHING.

The vial of concentrated washing solution is opened (22 ml) and poured into a 1000 ml measuring cylinder. Add 550 ml of distilled water and mix well. When used as a kit, the required amount of washing concentrate is separated and diluted 26 times with distilled water (in the ratio of 1 ml of washing solution concentrate + 25 ml of distilled water) [13].

IMPLEMENTATION OF REACTION.

1. Dilute the test samples to 41 times with buffer S011Z. Example: A 10 µl sample is filled with a buffer to dilute 400 µl. Milk samples and control whey cannot be diluted [14].
2. The required number of strips are placed on the frame, the test specimens are placed in monoplates, control serums in 4 holes (negative control in 3 holes, positive control in 1 hole).
3. Add 100 µl to the holes. negative and positive control serums and test samples (diluted whey (plasma) or unboiled milk) are added. Carefully, by circular motion, the tablet is mixed on the horizontal surface, glued with paper designed to glue the tablet.
4. Incubate at a temperature of + 370C for 60 minutes.
5. At the end of the incubation, the holes are washed 3 times by aspiration (e.g., water spray pump) or decantation. 250 mcl to all holes in each wash. is filled with washing solution, aspirated or decanted with circular motions along the horizontal surface. After each decantation, it is necessary to thoroughly remove the remaining liquid from the holes. To do this:
1. The wells are filled with 100 µl of conjugate.
2. Incubate at + 370C for 60 minutes.
3. The strips are washed 5 times with a washing solution.
4. Add 100 µl to all holes. from the substrate solution is added tetramethylbenzidine. The substrate solution should be added to the holes within 2 to 3 minutes. The tablet is incubated at room temperature, in a dark room (+18 ... + 250S) for 10 - 20 minutes until it turns blue.
5. All holes are filled with 100 µl of "stop" reagent in 2-3 minutes, the holes are painted orange.
6. The optical density is measured at a wavelength of 450 nm using a photometer, which scans the holes of the tablet vertically. The optical density should be measured within 15 minutes after the "stop" reagent has been added to the tablet holes.
7. The average optical density of a negative control is:

O3 (CN107BZ)3p + (O31 (CN107BZ)+O32 (CN107BZ)+O33(CN107BZ))/3.

The result of the analysis is considered reliable if:

- The optical density of the positive control is not less than 0.4 optical unit (OB).
- If the optical density of the negative control in all holes does not exceed 0.15 optical units.

8. Calculate the level of the limit value of Cutt, for which the negative control is added to the "optical" density of 0.5.

Cutt of +O3 (CN107BZ)3p + 0.5

9. The positivity index (PI%) is calculated for each sample tested, for which the optical density of the samples is divided by the amount of Cutt: **PI = example O3 / Cutt of**

Analysis of results:
ПИ › 1.1 The result is positive
ПИ ‹ 0.9 the result is negative

REFERENCES:

1. Авилов, В.М. Проблемы оздоровления крупного рогатого скота от лейкоза / В.М. Авилов, В.М. Нахмансон // Ветеринария.- 1995.-№ 11. - С. 3-6.
2. Аминева, С.П. Использование моноклональных антител для диагностики лейкоза КРС / С.П. Аминева, Л.А. Глобенко, В.И. Петров и др.// Материалы международной конференции, Харьков.- 1995.-С. 240-243.
3. Аминова, С.П. Моноклональные антитела для определения специфической активности различных антигенов вируса лейкоза КРС в ИФА / С.П. Аминова, Л.Б. Прохватилова, В.Н. Петров и др. // Сб. научи.трудов «Вирусные и микробные болезни с.-х. животных». - Владимир, 1995. - С. 36.
4. Белов, А.Д. О патогенезе лейкозов крупного рогатого скота /А. Д. Белов, Л.В. Рогожина, Г.В. Сноз // Ветеринария. - 1997. - № 12.-С. 16-19.

5. Бусол, В.А. О генетической предрасположенности к лейкозу, внутриутробной и горизонтальной передаче его крупному рогатому скоту / В.А. Бусол, Н.Н. Доронин, Г.Х. Субаев // в кн: Лейкоз крупного рогатого скота. - Рига: Зинатне, 1973. - С. 32-33.
6. Бусол, В.А. Организация научно-обоснованных противолейкозных мероприятий в неблагополучных хозяйствах Украины / В.А. Бусол,В. И. Цымбал, В.С. Ковалюшко // Материалы международной научной конференции, Харьков. - 1995. - С. 43^45.
7. Валихов, А.Ф. Биологические свойства вируса лейкоза крупного рогатого скота: диагностика и профилактика инфекции // Автореферат дисс. докт. биол. наук. М., 1992. - 46 с.
8. Валихов, А.Ф. Лейкоз крупного рогатого скота (вирусологические аспекты) / А.Ф. Валихов, В.П. Шишков, Л.Г. Бурба // М.: ВНИИТЭИСХ, 1980. - 78 с.
9. Галеев, Р.Ф. Характеристика гематологических показателей и серологических реакций у коров, спонтанно инфицированных вирусом лейкоза / Р.Ф. Галеев // II съезд гематологов и патоморфологов.-1985.-335 с.
10. Гулюкин, М.И. Исключить крайности в проведении противо-эпизоотических мероприятий при лейкозе крупного рогатого скота / М.И. Гулюкин // Ветеринарный консультант. - 2005а.- №13-14.-С. 4-6.
11. Гулюкин, М.И. Совершенствование профилактики и мер борьбы с лейкозом крупного рогатого скота / М.И. Гулюкин, Л.А. Иванова,А.В. Шишкин // Материалы международной учебно-методической и научно-практической конференции к 85-летию Академии. - М., 2004. -С. 242-248.
12. Гулюкин, М.И. Экспериментальное заражение кроликов вирусом лейкоза крупного рогатого скота / М.И. Гулюкин, Л.А. Иванова, Е.А. Шишкина, А.В. Шишкин, Л.Б. Прохватилова // Ветеринария. -2008.-№ 11.-С. 23-27.
13. AbabnehM.M. Detection and molecular characterization of bovine leukemia viruses from Jordan / M.M. Ababneh, R.K. Al-Rukibat, W.M. Hananeh, A.T. Nasar, M.B. Al-Zghoul // Arch. Virol. - 2012. - Vol. 157. -N. 12.-P.2343-2348.
14. Adam, E. Involvement of the cyclic AMP-responsive element binding protein in bovine leukemia virus expression in vivo / E. Adam, P. Kerk-hofs, M. Mammerickx, R. Kettmann, A. Burny, L. Droogmans, L. Willems// J. Virol. - 1994. - V. 68. - P. 5845-5853.
15. Avidan, O. The processivity and fidelity of DNA synthesis exhibited by the reverse transcriptase of bovine leukemia virus / O. Avidan, M.E. Meer,I. Oz, A. Hizi // Eur. J. Biochem. - 2002. - V. 269. - P. 859-867.