ISSN 0027-1314, Moscow University Chemistry Bulletin, 2018, Vol. 73, No. 3, pp. 135–137. © Allerton Press, Inc., 2018.
Original Russian Text © N.Yu. Saushkin, J.V. Samsonova, A.P. Osipov, S.E. Kondakov, E.S. Lysova, I.A. Elizarova, K.S. Khaertynov, E.A. Shuralev, 2018, published in Vestnik Moskovskogo Universiteta, Seriya 2: Khimiya, 2018, No. 3, pp. 235–239.

Strip-Dried Biofluids for the Detection of Specific Antibodies in Small, Infected Ruminants

N. Yu. Saushkin[a, *], J. V. Samsonova[a, b], A. P. Osipov[a, b], S. E. Kondakov[a, b], E. S. Lysova[c], I. A. Elizarova[d], K. S. Khaertynov[e], and E. A. Shuralev[c, d, e]

[a] *Department of Chemistry, Moscow State University, Moscow, 119991 Russia*

[b] *National University of Science and Technology MISIS, Moscow, 119991 Russia*

[c] *Department of Applied Ecology, Institute of Environmental Sciences, Kazan Federal University, Kazan, 420008 Tatarstan, Russia*

[d] *Laboratory of Biochemistry and Molecular-Genetic Analysis, Federal Center of Toxicological, Radiological and Biological Security, Kazan, 420075 Tatarstan, Russia*

[e] *Central Research Laboratory, Kazan State Medical Academy, Branch of the Federal State Budgetary Educational Institution of Further Professional Education, Russian Medical Academy of Continuous Professional Education of the Ministry of Healthcare of the Russian Federation, Kazan, 420012 Tatarstan, Russia*

**e-mail: sushk_90@mail.ru*

Received November 12, 2017

Abstract—The specific antibodies for viral arthritis-encephalitis and toxoplasmosis in goats is comparatively determined by the ELISA and latex agglutination reaction using strip-dried samples of serum and whole blood on a porous membrane carrier. It is shown that the use of strip-dried samples makes it possible to qualitatively and quantitatively determine specific antibodies and its results are completely consistent with those of the analysis of the liquid samples (serum). This sample preparation method can be used for the safe shipment of blood samples and following serological studies in epizootic monitoring.

Keywords: caprine arthritis encephalitis, toxoplasmosis, ELISA, latex agglutination test, strip-dried biofluids, dried blood spots (DBS)

DOI: 10.3103/S0027131418030069

INTRODUCTION

The dry blood spot technology (DBS) is widely applied in medical practice. When using this method of analysis, a patient's blood is applied onto the membrane carrier, dried, and then used for the further laboratory determination of various diagnostically significant substances and markers [1]. In particular, this technology is used for neonatal screening, pharmacokinetic studies, therapeutic drug monitoring, and other purposes. By using dry spots of blood and other biological fluids, it is possible to prevent intravenous intervention, reduce the patient's injury by collecting a small amount of capillary sampled material, and reduce the cost of the specialized transportation and storage of samples, since maintaining the so-called cold chain becomes unnecessary and the amount of transported material significantly decreases. Moreover, the technology for obtaining dry biological material can be used to create biobanks and makes it possible to carry out additional studies, which may be required to clarify the diagnosis or check questionable/controversial results, at any time.

In veterinary medicine, biological fluids are rarely used in dry form (on a membrane carrier) for sampling, transportation, and analysis and only a few studies have been published on this topic [2]. Barely any such studies have been performed in Russia, although we have recently published several papers demonstrating the successful use of dry samples of serum and/or blood plasma, whole blood, and whole milk [3, 4]. To prepare dry samples of biological fluids for their subsequent analysis and detection of DNA, antibodies, and hormones (cattle), we have taken a new approach based on the use of a thin strip of a fiberglass membrane. The technology of obtaining dry biosamples on membrane carriers is very promising for use in veterinary monitoring because it simplifies the task of collecting and transporting samples from farms (especially from remote farms) to a specialized analytical laboratory for performing analyses such as the haemagglutination inhibition reaction, latex agglutination (LA), enzyme immunoassay (ELISA), and the polymerase chain reaction. In particular, the approach of applying biological liquids on a membrane to obtain a dry sample can be used for large-scale sampling from cattle, small ruminants (SRs), pigs, and birds in order to perform subsequent serological studies and epizootic control aimed at the detec-

tion of antibodies against antigens of various pathogens and in the assessment of postvaccination immunity or detection of the genetic material of pathogens.

The aim of this study was a comparative use of the ELISA and LA methods for the detection of antibodies against antigens of SR pathogens in pairs of native and strip-dried samples of blood and serum prepared on a porous membrane carrier in the form of a thin strip.

EXPERIMENTAL

We used SR (goat) blood serum/whole blood samples obtained from small goat farms in the Republic of Tatarstan. Strip-dried samples were obtained by applying whole blood or serum on a membrane strip (by immersing the strip in a liquid sample) and then drying it. The carrier was a 0.5-cm wide marked strip fixed in a special card designed for the storage and transportation of biological fluids as dry blood spots (LLC Immunoved, Moscow). The dried samples were stored at 4°C with a desiccant in tightly sealed plastic bags.

To detect antibodies against the p28 antigen of caprine arthritis-encephalitis by ELISA, we used the MVV/CAEV p28 Ab screening reagent kit (IDEXX, France) for detecting antibodies specific to viral caprine arthritis encephalitis (and the Maedi-Visna disease in sheep) in the blood serum of SRs. A piece (0.5 × 0.5 cm) was cut according to the labeling from a membrane containing a dry sample and placed into in the well of a 96-well plate. The wells with samples were supplied with 200 μL of the sample dilution buffer. The plate was placed in a shaker (120 rpm) for 10 min. The plate was then covered with a lid and incubated at 37°C for 1 h. After the incubation, the solution was decanted and the membrane fragments remaining in the wells were removed with forceps. The further steps and the interpretation of the results were performed according to the manufacturer's instructions for the diagnostic kit.

The optical density of the samples was measured on a BioRad PR1100 spectrophotometer (BioRad, United States) at a wavelength of 450 nm. The coupling ratio of the antigen-to-serum antibodies (*S/P*, *%*) was determined for each sample using the following formula:

$$S/P = \frac{S - NC}{C - NC} \times 100.$$

Here, *S* is the average optical density of the sample, *PC* is the average optical density of the positive control, and *NC* is the average optical density of the negative control.

The measurement results were interpreted as follows: the result was considered negative at $S/P \leq 110\%$, suspect at $110\% < S/P < 120\%$ (in this case, it was recommended to re-examine the animal in two or three weeks), and positive at $S/P \geq 120\%$.

To identify antibodies against the antigens of the toxoplasmosis pathogen (*Toxoplasma gondii*) in SR (goat) blood by the test based on the latex agglutination reaction, we used the TOXOTEST-MT reagent kit (Eiken Chemical Co. Ltd., Japan) designed to detect antibodies in blood serum. A fragment (0.5 × 0.5 cm) was cut according to the labeling from a membrane with a dry sample and placed in an Eppendorf tube. The tubes with the samples were supplied with 120 μL of the buffer for the dilution of dry serum samples (or 60 μL of the buffer for the dilution of dry blood samples). Samples were mixed on a shaker at 120 rpm for 10 min. The diluted samples were used for further analysis. The analysis and interpretation of the results were performed according to the manufacturer's instructions for the diagnostic kit.

The measurement results were interpreted as follows. If the result was negative at the sample dilution of ≤1 : 16 (the precipitate of complexes had the form of a heap or a dot), the sample was considered to be free of antibodies against the infection. If the result was positive at the dilution of 1 : 16 (the precipitate had a form of an open umbrella) and negative at the dilution of ≥1 : 32, the sample was considered suspect (the animal should be re-examined in two or three weeks). If the result was positive at the dilution of ≥1 : 32, the sample was considered to contain antibodies.

RESULTS AND DISCUSSION

In this study, we determined specific antibodies against the antigens of the caprine arthritis-encephalitis virus and the *Toxoplasma gondii* protozoa by ELISA and LA, respectively. Viral caprine arthritis-encephalitis is an emergent infection. Its diagnosis is based on the detected antibodies. Recent studies [5, 6] indicate that this virus is circulating throughout the territory of the Russian Federation. Toxoplasmosis is also widespread and reported in all countries of the world. Its causative agent can parasitize in different hosts, including domestic and wild mammals, birds, and humans. The laboratory diagnosis of these diseases involves serological studies by immunochemical methods of analysis aimed at ensuring epizootic monitoring and control [7, 8]. The implementation of ELISA used in this study is based on the interaction of glycoprotein p28 of the caprine arthritis-encephalitis virus immobilized on a plate surface with the specific antibodies of the studied serum sample and the subsequent detection of the resulting complex with the conjugate (horseradish-peroxidase-labeled specific antibodies against SR IgG). Upon the addition of a substrate solution, the bound peroxidase catalyzes the oxidation of a chromogen (tetramethylbenzidine) by hydrogen peroxide. The intensity of color that develops in the wells of the plate is directly proportional to the amount of antibodies in the test sample.

Using ELISA, we analyzed 92 biosamples obtained by two different methods: strip-dried samples and liquid serum samples. Six of the strip-dried samples studied were obtained from whole blood. A total of 31 seronegative and 55 seropositive animals were identified in the analysis of dry serum. In the analysis of dry blood, we

Table 1. Results of analysis of liquid and strip-dried samples by LA method for antibodies against *Toxoplasma gondii* antigens

Sample	Maximum sample dilution that gave positive reaction		Interpretation of result	
	liquid samples (serum)	dry samples	liquid samples	dry samples
1–5	*	* (serum)	–	–
6	1 : 256	1 : 256 (serum)	+	+
7	1 : 256	1 : 256 (serum)	+	+
8	1 : 256	1 : 256 (serum)	+	+
9	1 : 64	1 : 32 (blood)	+	+
10	1 : 256	1 : 256 (blood)	+	+
11	1 : 64	1 : 64 (blood)	+	+
12	1 : 256	1 : 256 (blood)	+	+
13	1 : 16	1 : 16 (blood)	+/–	+/–
14	*	* (blood)	–	–

* No positive reaction was observed; + positive result; – negative result.

identified four seropositive and two seronegative animals. No samples showed suspect results. On average, the antibody content in the positive samples was at the level of S/P equal to or above 135.6 and 126.8% for the liquid and dry samples, respectively. The S/P value of most samples was higher than 450%. In the analysis of the negative samples, the coupling ratio of the antigen-to-serum antibodies was in the range of 0.4 to 59.3% (for the liquid samples) and 3.9 to 64.8% (for the strip-dried samples). In general, the interpretation of the results for dry biosamples fully agreed with that for the corresponding liquid serum samples.

The determination of antibodies against the *Toxoplasma gondii* antigens in the liquid and strip-dried samples of goat blood/serum was carried out using the LA reaction. This method is based on the interaction of the *Toxoplasma gondii* antigen immobilized on the surface of latex particles with the specific antibodies of the studied sample. When the reaction is positive, the resulting agglutinate precipitates in the form of an open umbrella. When the reaction is negative, it forms a heap or a dot. We analyzed a total of 14 samples of liquid serum, 6 corresponding to strip-dried blood samples and 8 corresponding to serum samples. When preparing a dry sample for analysis, we accounted for the fact that the formed elements of blood make up 40–45% of the total volume of the sample; and plasma, 55–60%. Thus, the membrane region with dry blood contains only half as much liquid blood (plasma) as the same-sized membrane piece with serum. Among the 14 studied samples, we identified 7 samples containing antibodies against the causative agent of toxoplasmosis in titers of 1 : 64–1 : 256 and one sample (no. 13) was classified as suspect. For the paired samples (liquid/dry), the interpretations of the results were in complete agreement.

CONCLUSIONS

Using the detection of antibodies against SR pathogens in the samples of goat serum and blood as an example, it was demonstrated that, along with the liquid samples, the dry samples can also be used to conduct serological studies, while maintaining the reliability of the results. Moreover, it is possible to analyze the dried samples of both serum and whole blood. The possibility of using dried whole blood samples, bypassing the serum isolation stage, for sampling, transportation, and analysis aimed at epizootic monitoring should be confirmed in the course of more comprehensive field studies.

ACKNOWLEDGMENTS

The work was financially supported by the Ministry of Education and Science of the Russian Federation as a based part of state assignment Organization of Scientific Researches (project no. 16.6548.2017/BY).

REFERENCES

1. Sharma, A., Jaiswal, S., Shukla, M., and Lal, J., *Drug Test. Anal.*, 2014, vol. 6, no. 5, p. 399.
2. Sun, D., Cho, Y.I., Comyn, P., and Yoon, K.J., *Vet. J.*, 2013, vol. 198, no. 2, p. 494.
3. Saushkin, N.Yu., Samsonova, J.V., Osipov, A.P., Kondakov, S.E., Khammadov, N.I., Usoltsev, K.V., Makaev, Kh.Z., and Chernov, A.N., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2016, vol. 71, nos. 5–6, p. 319.
4. Samsonova, J.V., Osipov, A.P., and Kondakov, S.E., *Talanta*, 2017, vol. 175, p. 143.
5. Balabanova, V.I. and Kudryashov, A.A., *Mezhdunar. Vestn. Vet.*, 2016, no. 4, p. 10.
6. Pokrovskaya, E.S., Shuralev, E.A., Mukminov, M.N., Elizarova, I.A., and Faizov, T.Kh., *Vet. Vrach*, 2015, no. 6, p. 16.
7. Panneum, S. and Rukkwamsuk, T., *Pol. J. Vet. Sci.*, 2017, vol. 20, no. 2, p. 347.
8. Wyrosdick, H.M. and Schaefer, J.J., *Anim. Health Res. Rev.*, 2015, vol. 16, no. 2, p. 150.

Translated by Yu. Modestova