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Comparative Research of Effectiveness of Cellulose and Fiberglass Porous Membrane Carriers for Bio Sampling in Veterinary and Food Industry Monitoring

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Abstract. The aim of proposed research is to study the applicability of fiberglass porous membrane materials in a new strip format for dried blood storage in food industry monitoring. A comparative analysis of cellulosic and fiberglass porous membrane materials was carried out to obtain dried samples of serum or blood and the possibility of further species-specific analysis. Blood samples of *Sus scrofa* were used to study the comparative effectiveness of cellulose and fiberglass porous membrane carriers for long-term biomaterial storage allowing for further DNA detection by real-time polymerase chain reaction (PCR) method. Scanning electron microscopy of various membranes - native and with blood samples - indicate a fundamental difference in the form of dried samples. Membranes based on cellulosic materials sorb the components of the biological fluid on the surface of the fibers of their structure, partially penetrating the cellulose fibers, while in the case of glass fiber membranes the components of the biological fluid dry out as films in the pores of the membrane between the structural filaments. This fundamental difference in the retention mechanisms affects the rate of dissolution of the components of dry samples and contributes to an increase in the efficiency of the desorption process of the sample before subsequent analysis. Detecting of pig DNA in every analyzed sample under the performed Real-time PCR as well as good state of the biomaterial preservation on the glass fiber membranes was clearly demonstrated. Good biomaterials preservation has been revealed on the test cards for 4 days as well as for 1 hour.

INTRODUCTION

Animal and plant infectious diseases cause serious problems in veterinary practice and plant growing. Efficient application of modern analytical techniques in agricultural and food analysis practice depends significantly from the possibility to store samples during their transportation from the point of collection (field, farm, etc.) to specialized laboratories. The sampling technique is a subject of specific interest in agricultural bioanalytics during last years, but publications with grounded choice of the best protocols are very rare, whereas the knowledge of industrial manufacturers has limited accessibility.

In 1963 Guthrie and Susi [1] suggested method of biomaterial storage for neonatal screening where blood samples were stored in the form of dried blood spots (DBS) on cellulose carrier. DBS technology is a microsampling alternative to traditional plasma or serum sampling. DBS technology has been applied to diagnostic screening in drug discovery, for pharmaco- or toxicokinetic evaluation nonclinical, and clinical settings. This method is a good alternative to the classical ones due to relative noninvasiveness, easy realization, simple sample treatment and storage, very low volumes of biomaterials applications [2]. In medical practice this approach was lately effectively adapted for infectious disease diagnostics [3,4], including human immunodeficiency virus [5-8], hepatitis [9-12], and flaviviridae [13]. Moreover, this technique is suggested as

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the major one for creating biobanks of humans [14]. However, in spite of all the apparent advantages, DBS method is still rarely used in veterinary medicine and food analysis.

Classical veterinary may use DBS method for detecting pregnancy in cows and heifers [16-17]. Smith and Burgoyne [18] reported good results of nucleic acids long-term storage on cellulose fiber matrices for further DNA analysis and pathogens detection as exemplified by blood samples taken from chicken (*Gallus gallus*), cow (*Bos taurus*), western shingleback *Tiliqua rugiosa* and Australian pelican (*Pelecanus conspicilattus*). Study performed on goat blood samples showed that DBS method was more efficient for trypanosomiasis diagnostics employing PCR analysis than any conventional approach to blood samples collection and storage [19]. Rosypal et al. [20] estimated DBS method feasibility for canine leishmaniasis diagnostics using anti-*Leishmania infantum* immunochromatographic tests and demonstrated that the biomaterials introduced into the matrix after 30 days retained 100% sensitivity and specificity compared to standard frozen blood method. Furthermore, the results obtained by Andersson et al. [21] allow one to assume that DBS should be used as method of choice for immunoenzymatic assay aimed at Aleutian disease of mink's diagnostics as it combines simplification of diagnostic procedures with retaining sensitivity and specificity of the biomaterial.

According to Lehner et al. [22] the aforementioned technique is highly suitable for population studies including the environmental assessment of heavy metals impact. It is also important to note that DBS method requires comparatively small volumes of blood that makes studies of such animals as birds, reptiles, amphibia and small mammals more accessible [22, 23]. As exemplified by experiments involving laboratory rats and mice, DBS technique in preclinical studies can lead to considerable decrease in numbers of used animals without any loss of the experimental results quality [3, 24].

Development of glass fiber matrices that surpass cellulose analogues in almost every aspect has become a next step for DBS technology. At present a number of research papers reveal positive characteristics of glass fiber as matrices material for biomaterial storage and transportation in toxicology [26] and medical diagnostics [27]. Unlike cellulose materials glass fiber matrices do not irreversibly adsorb biological fluids components and don't entrap them inside the fibers. At the same time the properties of modified glass fiber matrices as high breaking strength, good wettability and capacity make them an attractive material for researchers and future consumer [25].

Earlier in our laboratory a new sampling format for preparation of dried spots on the strips of membrane made of porous fiberglass material was proposed [28] and the samples were successfully used for the detection of proviral DNA of leukemia virus in cows by polymerase chain reaction (PCR) and ELISA methods [29], for analysis of progesterone in dried samples of milk by ELISA in order to identify nonpregnant cows [17] and for quantitative determination of some low and high molecular hormones and proteins in serum for medical diagnostics [30, 31]. Reliable preservation of important biomaterial characteristics makes this method very promising for a variety of applications in veterinary and food quality analysis. For example, present ecological situation and anthropogenic impact lead to active pollution of livestock food and, as a consequence, accumulation of a wide variety of toxicants, including heavy metals, in animal products. In this regard use of matrices for dry storage of biosamples can considerably simplify biosampling process for stock-breeders for regular control over toxic substances, including heavy metals, content both in vegetable matter and in animal products [22]. Beside inorganic pollutants wide spread of virus infections is another problem of livestock and plant growing sectors. Late diagnostics of virus infections results in livestock deaths, crop decrease and general financial losses. The technique described in this paper is effective for sample storage with further foreign DNA detection by means of PCR analysis in plant [32-34] and animal [19, 21, 35-36] biosamples. Due to active development of genetic engineering a demand arose in agriculture and food industry for control over quantity of genetically modified products. Dry biosamples storage method may be very convenient for this purpose and its successful use in combination with PCR analysis already received experimental validation [32, 37]. Another promising application for dry biosamples storage method is assessment of antibiotic substances and hormonelike materials remaining content in food. The accumulated experience together with experimental data prompt suggestions that this method may become the main one for biosamples storage and transportation for further screening [38, 39]. Furthermore, dry biomaterial storage method can be used in test systems to determine the type of meat (e.g. pork) in products for people who avoid eating some types of meat for certain reasons.

The aim of this paper is to estimate the feasibility of fiberglass strips with dried blood samples of *Sus scrofa* for long-term biomaterial storage allowing for further swine DNA detection by real-time polymerase chain reaction which may be useful in food industry applications, compared with cellulose carriers. For example, at present, in many countries special attention is paid to the production of halal food products, including products that do not contain pork. In this paper it is shown that the method of sample preparation using dried blood stains technology can be used to establish the presence in the samples of the species-specific pig DNA.

EXPERIMENTAL PART

Test cards for dried samples

For collection of dried biomaterials we used special test cards with fiberglass membrane strip commercially produced by Immunoved Ltd (Russia) (Fig.1a) and 903 “Protein Saver” cellulose filter paper cards (Whatman, USA) (Fig.1b).

To study the morphology of the membrane supports a JEOL JSM6610LV scanning electron microscope was used in this work. Samples of the membrane support were pretreated prior to imaging. A sample with sides of approximately 5 mm was cut from a sheet of membrane support. The sample was glued to a conductive carbon double sided adhesive tape, which was in turn placed on a sample stage. The next step involved coating the membrane support sample with a conductive layer. The coating was conducted using a tabletop magnetron sputter coating instrument JFC1600 Jeol. Platinum was used as a coating material with an ionization current of 30 mA and a coating time of 40 s. The field of view at different magnifications (×50, ×100, ×300, ×500) was used.

Biosamples

Studies were carried out using the blood samples of Duroc pigs - the most popular on the Russian market. Blood was obtained from the pigs of LLC "Agrobelgorye Group of Companies". The blood samples (1 ml) were collected in EDTA K3 (Apexlab) evacuated blood collection receptacles and stored in the refrigerator at +4 ^{o}C. 400 μl of blood was transferred by semiautomatic dosator to a standardized 1.5 ml Eppendorf polypropylene test tube. To apply the biomaterial, the first part of the membrane was placed into a whole blood sample and incubated to wet the strip completely; the membrane was then removed and air dried at room temperature for 1.5–2 h. The dried samples were stored at 4°C in tightly sealed plastic bags with a dessicant.

DNA Purification

DNA purification was carried out with a genomic DNA purification from biological material using *K-Sorb-100* columns (*Syntol*, Russia, catalogue number EX-514-100) designed for DNA purification from blood, saliva and packed cells in physiological solution.

Polymerase Chain Reaction

Three protocols were used for real-time polymerase chain reaction (Real-time PCR) analysis of the blood samples:

1) The reaction was performed with the native biomaterial.

2) The biomaterial was applied onto a test card, after 1-hour drying the material was washed from the card, the reaction was performed.

3) The biomaterial was applied onto a test card, after 1-hour drying the material was kept in the thermostat at +42°C for 4 days. After that the material was washed from the card, the reaction was performed.

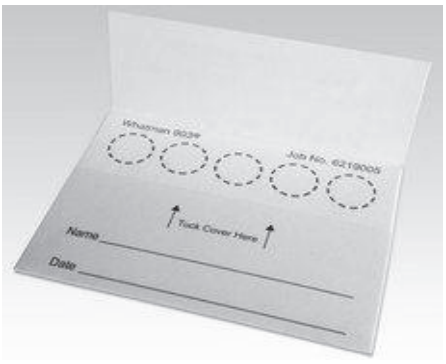
The aforementioned procedures were performed for ten blood samples.

Real-time PCR was performed using ABI StepOne Plus (Applied Biosystems, USA) thermocycler. Polypropylene 0.1 ml test tubes MicroAMP (Applied Biosystems, USA) were used for thermal cycling.

Standardized test-system Sus scrofa Ident RT (Syntol, Russia, ID-203) was employed for identification of the purified DNA. This test-system is designed for detection of species-specific pig (*Sus scrofa*) DNA by means of real-time PCR method.

RESULTS AND DISCUSSION

DBS cards of conventional format are manufactured of cellulose fibers and samples are applied dropwise onto marked round parts of a special card. After drying paper discs with dried sample are punched. In this work we used a new sample preparation format for sampling, storage, transportation and analysis of dry blood samples. A thin marked membrane made in the form of a strip of porous hydrophilic fiberglass material was used as the carrier of biological material (Fig.1).



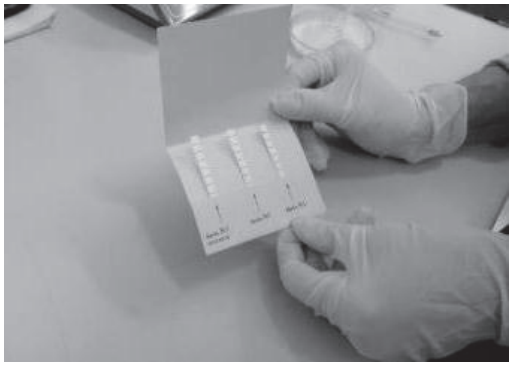
(a)

(b)

FIGURE 1. (a) Appearance of a standard card from Whatman 903 cellulose filter paper used to obtain dried blood spots; (b) Cards of sets of membrane carriers of the developed new format with three fiberglass strips

This format of sample pretreatment based on DBS technology includes application of liquid sample onto the end of marked narrow strip of fiberglass membrane material (0.5 cm width). In comparison with the traditionally used cellulose support, a fiberglass membrane has a number of mechanical and structural advantages (higher strength and deformation resistance, solid fiber structure etc.) The applied sample distributes along a strip evenly and after drying a square part of membrane (0.5x0.5 cm) can be cut by scissors and analyzed by PCR or ELISA methods. One strip with applied sample gives up to 8 parts of carrier with equal amount of dried sample. There is no need to saturate carrier with visualizing agents to collect non-coloured bio-fluids as soon as migrated biofluid saturates the defined amount of marked parts (squares) of the strip.

The main physical working characteristics of the fiberglass membranes in the cards are given in Table 1.

TABLE 1. Characteristics of fiberglass membrane strip of Immunoved-SPK test cards

Thickness	Specific weight	Soaking time	Specific water loading of membrane
0.43mm	75 mg/cm^2	3 sec/ 2 cm	48 mg/cm^2

In Figure 2 the scanning electron microscopy images of blood serum samples obtained on standard cellulosic carrier and in the hydrophilized fiberglass-based membrane carrier are shown to confirm the mechanism for retaining dry serum samples. The data obtained on scanning electron microscope for two membrane supports clearly demonstrate the structural differences between the cellulose and fiberglass membranes (Fig. 2).

In contrast to hollow cellulose fibers (parts c and d in the Figure 2), the solid structure of the fiberglass strands makes it impossible for a biological liquid to penetrate the fibers (parts a and b in the Figure 2) and also increases the efficiency of elution of the analyzed components from the membrane. Moreover, the structure of the fiberglass membranes makes it possible to eliminate the chromatographic effect of the distribution of components during the biological liquid absorption and to overcome the difficulties in the quantitative analysis of whole blood that are associated with the hematocrit effect and are typical of cellulose membranes.

Electronic microphotographs of native and serumed blood and various membranes indicate a fundamental difference in the form of dried samples. Membranes based on cellulosic materials sorb the components of the biological fluid on the surface of the fibers of their structure (Figure 2d), partially penetrating the cellulose fibers, while in the case of glass fiber membranes the components of the biological fluid dry out as films in the pores of the membrane between the structural filaments (Figure 2 b). This fundamental difference in the retention mechanisms affects the rate of dissolution of the components of dry samples and contributes to an increase in the efficiency of the desorption process of the sample before subsequent analysis.

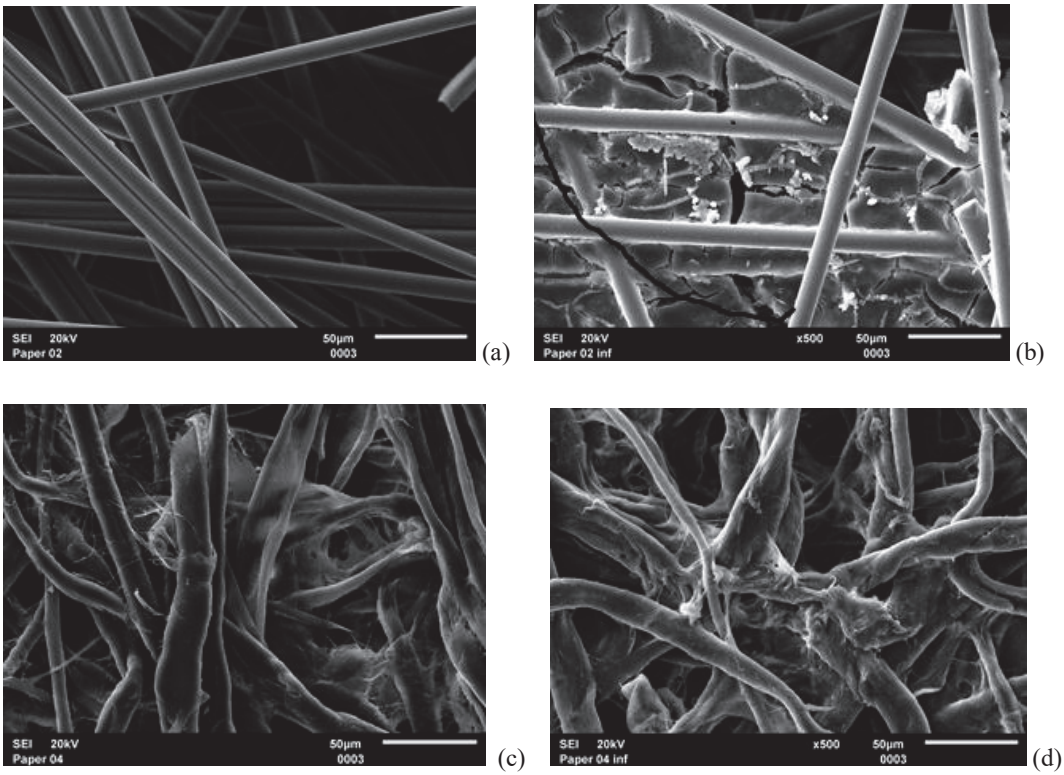


FIGURE 2. Scanning electron microscopy images of membranes: a—fiberglass membrane without a sample; b—fiberglass membrane with a dried blood sample; c—cellulose membrane without a sample; and d—cellulose membrane with a dried blood sample

The proposed method of applying blood or other biological fluid to a porous fiber glass membrane carrier in a strip format is based on the inclusion of liquid in the pores of a hydrophilic inert carrier by capillary forces. The use of a standardized porous membrane carrier allows the inclusion in its pores of strictly fixed volumes of liquids proportional to the surface area of the membrane carrier or strip, and therefore differences in viscosity, blood hematocrit, and other properties should not affect the quantitative characteristics of the assay. In other words, equal areas of a porous membrane carrier contain the same volume of liquid being analyzed, and consequently, it is possible to create reproducible systems for performing quantitative analysis.

The principal difference between the proposed format of cards and usual is a new composition based on glass fiber, specially modified to give it hydrophilic and bactericidal properties [25]. A distinctive feature of such carriers before cellulose is inertness in relation to the components of biological fluids and almost complete desorption when the samples dried on them are washed with buffer solutions.

Detecting of pig DNA in every analyzed sample under the performed Real-time PCR as well as good state of the biomaterial preservation on the glass fiber membranes was clearly demonstrated (Fig. 3).

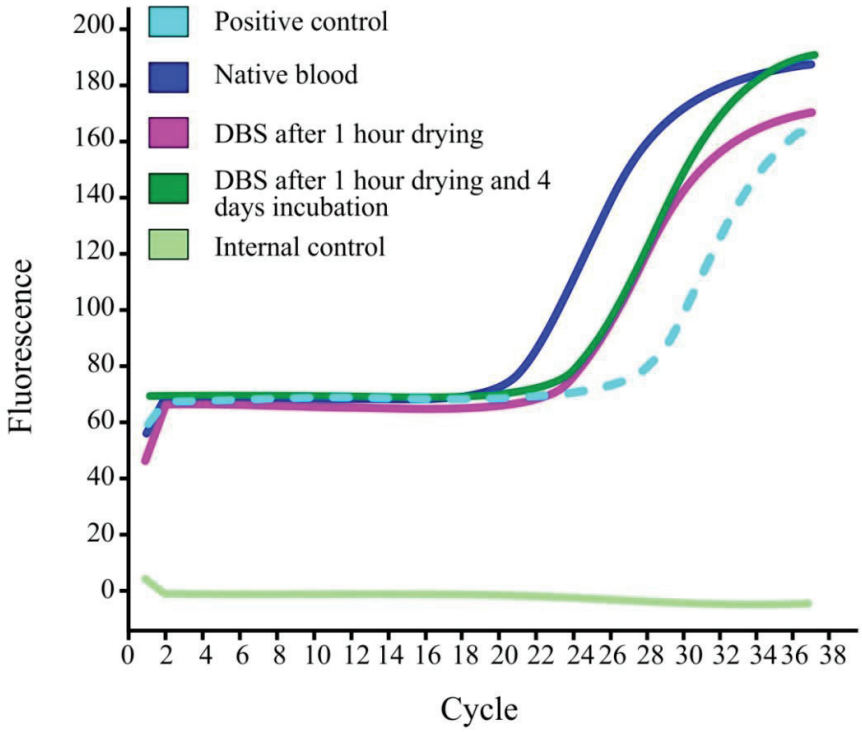


FIGURE 3. Kinetic curves obtained during real-time PCR: pig DNA is detected in every sample

The performed experiment allowed to confirm that domestic pig (*Sus scrofa*) DNA is preserved on the test cards for 4 days as well as for 1 hour. The obtained results demonstrate their applicability for biological materials storage for further detection of various agents' DNA by PCR method.

Modern veterinary uses whole biological fluids as main diagnostic subjects. As a rule, using such analytical material is handicapped by a number of aspects: firstly, the samples require freezing and throughout their storage temperature regime must be rigorously observed which is often problematic under the field conditions; secondly, high-quality analysis demands large volumes of biomaterial especially for repeated tests which is not always possible and thirdly, transportation and storage of frozen samples is always financially challenging.

CONCLUSION

In our research work we once more displayed advantages of biomaterial storage in the form of DBS using glass fiber matrices for this purpose. This technique ensures genetic material preservation and shows consistency of recorded PCR results.

Electronic microphotographs of various membranes - native and with blood samples - indicate a fundamental difference in the form of dried samples. Membranes based on cellulosic materials sorb the components of the biological fluid on the surface of the fibers of their structure, partially penetrating the cellulose fibers, while in the case of glass fiber membranes the components of the biological fluid dry out as films in the pores of the membrane between the structural filaments. This fundamental difference in the retention mechanisms affects the rate of dissolution of the components of dry samples and contributes to an increase in the efficiency of the desorption process of the sample before subsequent analysis.

Real-time PCR performed for all the samples detected pig DNA in every sample as well as good state of the biomaterial preservation on the glass fiber membranes was clearly demonstrated. The performed experiment allowed us to confirm that domestic pig (Sus scrofa) DNA is preserved on the test cards for 4 days as well as for 1 hour.

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