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REVIEWS

Dried Samples of Biological Fluids on Porous Membranes as a Promising Sample Preparation Method for Biomedical and Veterinary Diagnostics

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Abstract—The sample preparation method for liquid biosamples dried on a carrier (dried blood spot technology) has been increasingly used in various fields of bioanalysis, pharmacokinetics, therapeutic drug monitoring, toxicokinetics, metabolomics, and disease diagnostics over the past 20 years. The dried blood spot technology involves the application of a sample directly to the carrier; after drying, the sample is analyzed using modern analytical, immunochemical, and genetic methods. The advantages of this sample preparation method include minimal invasiveness; small sample volumes; ease and cost-effectiveness of sampling, transportation, and storage of samples; and high stability of analytes. The review considers the main aspects of the application of the sample preparation of biological fluids, suspensions, tissues, and organs in the form of dried spots on porous membrane carriers in analytical practice. The principles of using membrane carriers in order to obtain biological samples in the form of dried spots, approaches to their analytical application, interfering factors and limitations associated with the rheological properties of blood, types of devices for taking blood and other samples, and the current state and prospects for the development of dried spot technology are described.

Keywords: biological fluids, dried blood spots, porous membrane carriers, extraction

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The preparation method of blood samples in the form of spots dried on a carrier—the dried blood spot (DBS) technology—has become widespread since the 1960s when dried blood spots of newborns obtained on filter paper were successfully used to detect the hereditary disease of phenylketonuria by determining the marker phenylalanine [1]. Using this approach, Dr. Robert Guthrie identified 39 cases in 400000 newborns at an early stage, and this ultimately led to the development of neonatal screening and the introduction of the DBS technology into medicine. To date, the sample preparation of biological fluids in the form of dried spots is the most important tool for neonatal screening and detection of rare genetic diseases of newborns, and it is also widely used in various bioanalytical studies, primarily, in preclinical drug trials and medical, toxicokinetic, and pharmacokinetic studies and in clinical pharmacology; samples dried on a carrier are also used in forensic, doping, and environmental examinations and in the creation of biobanks [2–11]. Both low-molecular-weight substances and protein molecules, such as antibodies and DNA/RNA, are determined in dried spots of biological fluids. The use of the DBS technology makes it possible to reduce the invasiveness of blood sampling, that is, injury to the patient, and to decrease the mortality of experimental animals in preclinical studies because only one drop of blood (30–50 μL) is required to obtain a dried sample in the form of a spot. Dried samples are highly stable, and they do not require a cold chain to be transported to the site of analysis. For this reason, this approach was successfully used in the remote diagnostics of human diseases and the monitoring of epidemiological situations, for example, in the detection of the human immunodeficiency virus (HIV) in African countries, where hot climate and insufficient laboratory support make it difficult to work with liquid samples [12–14]. This approach makes it possible to simplify the storage and transportation of biosamples because dried samples can be stored and/or sent to the site of laboratory analysis at ambient temperature, including elevated temperatures, for several days, while maintaining the stability of components adsorbed onto the membrane carrier. The risk of biological contamination is reduced in operations with dried samples; in addition, dried samples are easier to dispose than liquid ones after analysis. Dried blood spots can be easily obtained by the patient independently because they do not require special skills in working with a sampling kit, and dried

samples can be sent to the laboratory, for example, by mail, without unnecessary transportation costs. Due to their advantages, bioanalytical methods based on the DBS technology have already become a serious alternative to traditional analysis of liquid venous blood or serum. The use of biosamples in the form of dried spots is currently successfully extended not only to whole blood but also to other biological fluids, such as saliva, plasma, synovial fluid, urine, and milk [15]. These dried spots are commonly referred to as matrix spots. Target analytes are determined in dried spots using various analytical techniques such as gas chromatography (GC) or liquid chromatography (LC) combined with mass spectrometry (MS), immunochemical methods such as enzyme-linked immunosorbent assay (ELISA), methods based on the principle of agglutination, polymerase chain reaction (PCR), etc. [16–20].

Interest in the DBS technology, which has been rapidly developing in the last 10–15 years, has not weakened so far. According to Scopus, more than 400 scientific articles on this subject matter have been published annually since 2014, and about 10% of them are review articles (Fig. 1a). In Russia, according to Scopus, the number of scientific publications on the DBS subject technology is much lower compared to that in many other countries (Fig. 1b). So far, insufficient attention has been paid to this subject matter in Russian science, and the use of the DBS technology in actual practice is limited.

PRINCIPLE OF THE METHOD OF BIOLOGICAL SAMPLE PREPARATION IN THE FORM OF DRIED SPOTS ON POROUS MEMBRANES

Sample preparation in the form of dried spots is based on the drop application of a biological fluid or suspension or obtaining an imprint of an organ or tissue on a special absorbent membrane and the subsequent drying of the sample. The porous membrane material, which consists of intertwined fibers, absorbs the applied sample due to capillary forces according to its sorption capacity. In the classical version, a card for sampling whole blood is an envelope with a cellulose membrane in the form of a rectangle with designated round areas for sample application inside (Fig. 2). The membrane material may contain pre-applied reagents to increase the safety of proteins or nucleic acids, for example, lysing and chelating agents and detergents [21, 22].

In the sampling of blood, it is applied dropwise to the center of a circle according to the rule: one drop per circle. As a rule, blood is taken from the finger, the heel, and the ear or tail in adult patients, newborns, and animals, respectively. After applying a biosample, the sample card is dried at room temperature; thereafter, the dried sample is ready to be sent to the laboratory. In addition to dropwise application, it is possible to apply a required volume of biological fluid with a semi-automatic dispenser and to obtain an imprint of the test organ (for example, the spleen) or tissue by contact with an absorbent membrane material. Thus, such cards for obtaining dried spots are a universal means for storing and transporting a variety of biosamples.

To perform analysis in the laboratory, a membrane section in the form of a disk with a diameter of several millimeters is cut out from a dried spot, for example, using a special hand-held device (a medical puncher). The dried sample is then eluted into solution to perform the appropriate analysis. The development of modern analytical systems is aimed at automating the processes of sample preparation, extraction, and analysis of samples. Currently available automated stations can simultaneously work with dozens of DBS cards and carry out their identification, sorting, separation of sections with samples, subsequent extraction, and addition of an internal standard (IS) [23]. These autosamplers in combination with analytical LC–MS instruments make it possible to rapidly quantify diagnostically significant substances in blood [24–26].

ANALYTICAL ASPECTS OF THE DRIED BLOOD SPOT TECHNOLOGY

It is of great importance for drug development and clinical research to understand how the target analyte is distributed between plasma and red blood cells. For the DBS technology, it is important to know how analyte concentrations in dried spots will correlate with the actual concentrations in blood plasma/serum because whole blood, which contains both plasma and blood cells, is applied to the carrier and the blood cells undergo lysis in the course of drying the sample on the membrane. Historically, plasma or blood serum separated from cells is used for analytical purposes in medicine and veterinary medicine. Erythrocytes play an important role in analyte distribution, and they can influence the data obtained in pharmacokinetics and pharmacodynamics [27]. If an analyte in blood occurs not only in plasma but also in erythrocytes, its concentration determined in a dried spot will be higher than that in plasma. This distribution of the analyte depends on its physicochemical properties, and it should be confirmed in the parallel correlation measurements of dried blood samples and liquid plasma/serum samples with the introduction of a correction factor, if necessary.

Extraction of dried spots from the carrier. The extraction of a sample adsorbed onto the membrane material is one of the most important preparatory stages before quantitative analysis. The efficiency of extraction directly affects the recovery of a substance and, ultimately, the sensitivity of methods to the target analyte. Moreover, the step of extraction is important when an IS is added and in extracting DBS samples

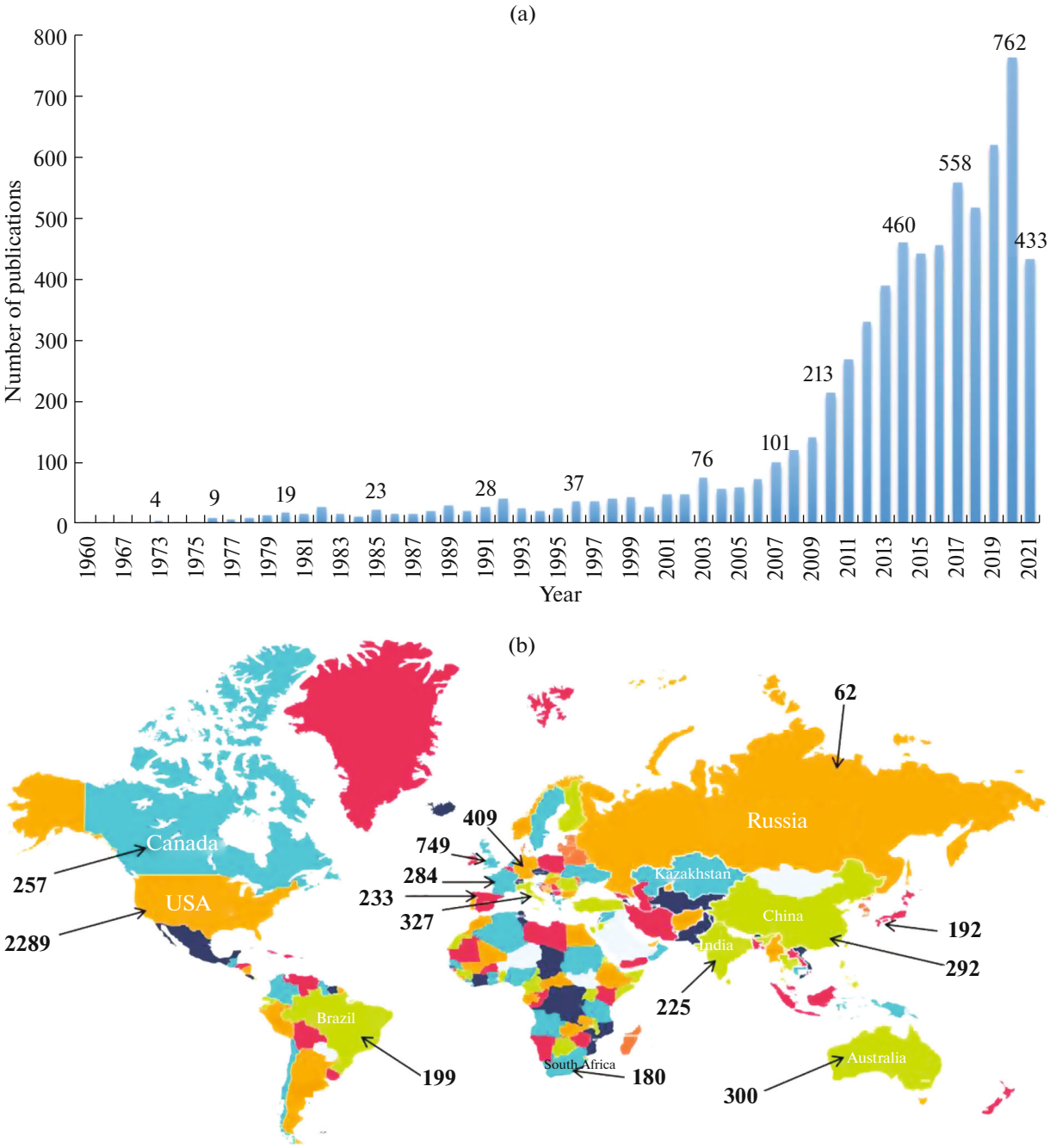

Fig. 1. (a) Distribution of publications on the DBS technology over years according to Scopus data and (b) the numbers of publications issued from 2000 to 2021 in different countries.

with organic solvents and mixtures for analysis performed by instrumental methods [28, 29].

In general, methods for extracting biosamples in the form of dried spots are subdivided into two groups: offline and online methods. In the offline extraction, all of the necessary stages of dried sample recovery by transferring the components sorbed on the carrier into a solvent are carried out manually. These are the separation of a required section of the membrane with a dried spot, the addition of an extracting solution with an included IS, centrifugation, and the injection of the resulting extract into the chromatographic system. Sometimes, evaporation, repeated dissolution in a solvent more suitable for a particular system, etc., are also required. Offline extraction is an effective procedure, but it is labor-intensive and time-consuming, and this limits the widespread use of the DBS technology in non-instrumental methods of analysis, such as immu-

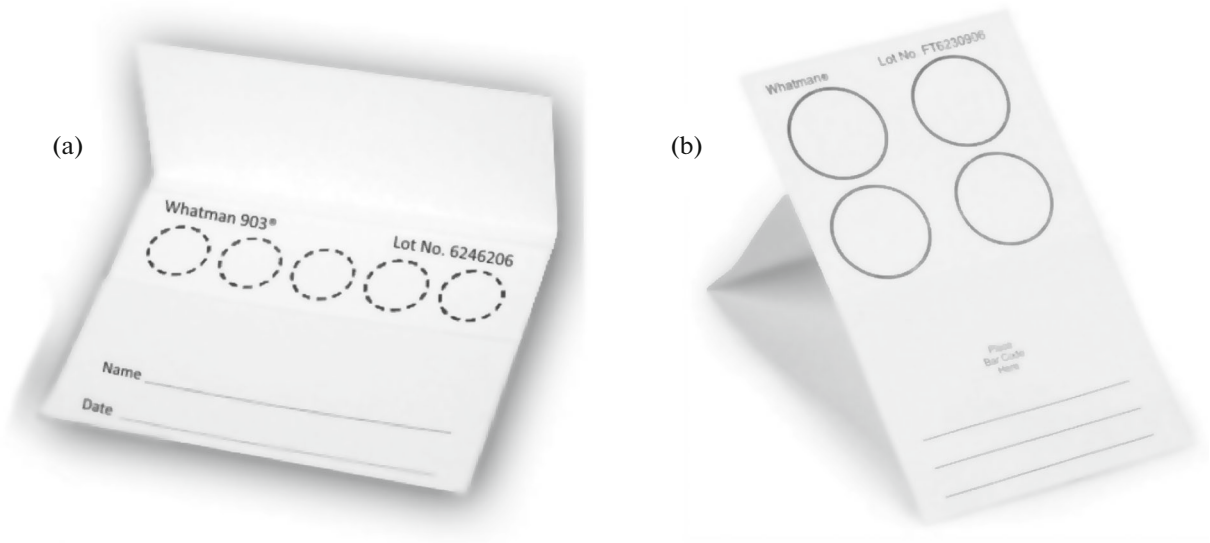

Fig. 2. (a) Classical version of the Whatman 903 card for the colllection of dried blood spots and (b) the FTA card (Whatman, the United States) for storing blood spots for PCR analysis.

nochemical methods and PCR. Moreover, as a rule, the extraction of analytes into a buffer solution is used in the former case, and standard extraction methods are used for the separation of nucleic acids and the subsequent PCR [22, 30, 31]. Unlike the offline extraction, online extraction is a process that reduces time consumption by automating and simultaneously preparing several samples for analysis. Online extraction became possible due to the development of appropriate instruments for automatic DBS sample preparation. CAMAG (Switzerland) produces automated sample preparation systems equipped with a barcode reader and a built-in washing station with the ability to add an IS, which can be coupled to LC–MS systems [23]. This system is capable of processing a set of 500 DBS cards. Another instrument from Prolab GmbH (Reinach, Switzerland) is capable of applying various extraction protocols due to the possibility of clamping cards and passing a solution under pressure to 200 atm through the blood spot. Automated stations for the extraction of DBS samples are combined with solid phase extraction (SPE). Spark Holland (Emmen, the Netherlands) presented a modified unit that does not require preliminary separation of the extracted sample using LC. The unit can perform flow-through pre-extraction by passing the eluant through the spot and then directing the flow to the SPE–MS/MS system. This unit can process about 20 samples per hour with high accuracy and reproducibility.

Ideally, analysis based on DBS should be carried out without the preliminary steps of cutting out a part of the membrane with a sample and elution, for example, based on direct surface MS analysis. The direct surface MS analysis makes it possible to form charged ions at room temperature and normal pressure and then send them to a mass spectrometer for quantitative determination [32]. The direct surface MS analysis includes desorption electrospray ionization (DESI) due to the bombardment of a surface with charged microdroplets with the formation of drops containing the target analyte, which evaporate in a heated capillary system and are detected in a mass analyzer. PaperSpray, DESI (Prosolia Inc., the United States), and direct analysis in real time (DART) are the most common ionizing analyzers [32]. The DART analysis is based on the direct effect of ionizing gases on a dried analyzed sample with the exception of the stage of preliminary extraction of biological material from the carrier. Such a method can significantly shorten the analysis time, but it still ranks below traditional MS methods in terms of sensitivity and reproducibility of results.

Methods for the analysis of dried spots of biological samples. In most cases, the available volume of test material is sufficient for qualitative and quantitative analysis; however, it should be taken into account that the used method of DBS analysis should be sufficiently sensitive due to a small volume of the test sample [2, 4, 6, 9]. GC and LC MS analysis, atomic absorption spectroscopy, and inductively coupled plasma MS are used to quantify the target analyte in dried spot samples. LC in combination with various detection techniques is most often used for DBS analysis because most of the analytes are organic substances, drugs, their metabolites, and endogenous compounds. The combination of GC with MS is used to analyze DBS samples in neonatal and metabolic screening [33, 34] and in the determination of amino acids, hormones, and other metabolites. To determine

a wide range of substances, MS with matrix-assisted laser desorption/ionization (MALDI–MS) is also used. In this case, the preliminary separation of samples using LC is usually not required; because of this, the duration of the analysis is shortened. It was found that semiquantitative and, in some cases, quantitative determination can take a uniquely short time of about 5–10 s per sample [35, 36].

As noted above, most of the methods using DBS involve the determination of low-molecular-weight substances, for example, disease biomarkers, drugs, prohibited substances, etc. [2–11]. Dried samples are used much less frequently for the determination of macromolecular substances. Kehler et al. [37] compared the efficiency of LC with tandem MS detection (LC–MS/MS) for the determination of a protein molecule with immunoassay, the gold standard for the detection of high-molecular-weight proteins. They concluded that the use of MS for the quantitative determination of large protein molecules is possible, but the sensitivity of the method in the DBS analysis is lower by a factor of about 20 than that in immunochemical analysis. Note that immunochemical methods are used much less frequently for the analysis of dried spots. This is primarily due to the complexity of the analysis and a large number of dried sample preparation procedures performed in manual mode because the use of instrumental methods for the quantitative determination of macromolecular substances is limited. In addition, quantitative analysis is difficult due to the complexity of the blood composition and the distribution of substances between plasma and blood cells [27]. However, ELISA and qualitative or semi-quantitative methods based on agglutination are used in serological studies, for example, in the monitoring of infectious diseases in the populations of agricultural and wild animals and birds [38, 39]. The PCR method is used to identify the desired nucleic acid fragments in dried samples, and a sample of any origin (a biological fluid, extract, suspension, tissue, or organ) applied and dried on a carrier is suitable for extraction and the subsequent amplification [21, 22].

Stability of components to be determined in dried samples. In most cases, an increase in the stability of the determined components of biological fluids after drying on a membrane carrier is one of the advantages of sample preparation in the form of dried spots. For example, the observed concentrations of amino acids stored in DBS samples at room temperature only slightly decreased in a range from 3.1 to 7.3% annually [40]. It was found that degradable substances such as acylcarnitines were stable for at least 330 days upon storage at –18°C. When the dried samples were stored at room temperature, the half-life of acylcarnitines ranged from 161 to 990 days [41].

Antibodies are sufficiently stable when stored in carrier-dried blood, serum, or plasma. Thus, DBS samples obtained from wild reindeer in the field showed identical results upon the determination of antibodies to pathogens of infectious diseases during two years of storage at room temperature [31]. DNA- and RNA-containing viruses can also be kept dried for a long time. Cardona-Ospina et al. [30] described numerous examples of the use of special FTA cards (Whatman, the United States) impregnated with lysing and chelating agents for cell lysis and an increase in the preservation of nucleic acids of pathogenic viruses during storage. Thus, Michaud et al. [42], who used small ruminant distemper virus as an example, demonstrated that single-stranded viral RNA of the causative virus can be detected in DBS samples after at least three months of storage at temperatures typical for tropical countries. Moreover, the proviral DNA of the African swine fever virus was detected in the DBS analysis even after nine months of storage at 37°C. Madhanmohan et al. [43] determined the foot-and-mouth disease virus (FMDV) RNA in dried epithelium samples from the tongue and foot of infected cows, which were sent by mail without refrigeration along a long route from the sampling site to the laboratory after a rainy period and during summer in India. They found that, regardless of temperature conditions (21–45°C) and environmental humidity (20–100%), the genome and serotype of FMDV were determined in all samples 22–56 days after the sampling.

The use of membrane carriers for the storage of dried samples improves the stability of many biomolecules due to dehydrating the sample and, consequently, minimizing enzymatic and hydrolytic processes. For example, the substance lopinavir was significantly better preserved in DBS samples for two years of storage than in frozen plasma [36]. Alfazil and Anderson [44] used benzodiazepines, which are determined in forensic medicine, as an example to demonstrate an increase in the stability of analytes during storage on a carrier. In some cases, it has been noted that the storage stability is affected by the presence of a stabilizing agent, for example, EDTA, in the membrane carrier [45]. Summing up, we can conclude that the DBS technology offers a simple and efficient way for the transportation and storage of biological samples by reducing the cost of moving samples to the site of analysis and storage, and it reduces the requirements for compliance with temperature standards. The stability of a particular substance and the possibility of its analysis in a dried matrix spot should be studied separately; however, in most cases, the transportation and short-term storage of dried samples can be safely carried out without refrigeration.

PROBLEMS AND LIMITATIONS IN THE USE OF SAMPLE PREPARATION IN THE FORM OF DRIED SPOTS

Effect of the blood sample's hematocrit value. The variability of whole blood hematocrit levels from sample to sample is currently a serious limitation of the use

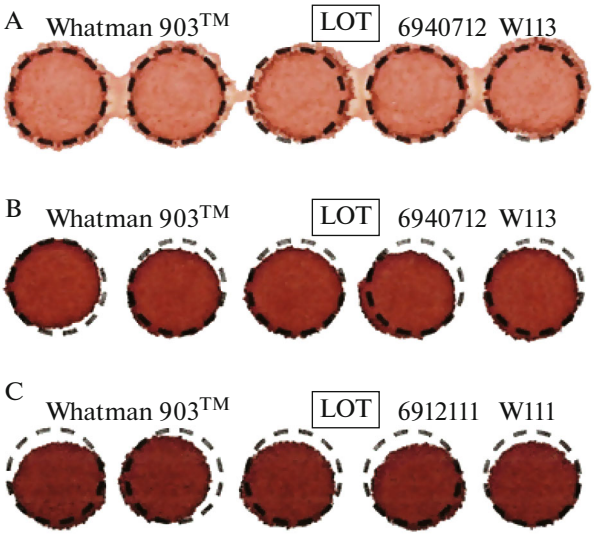

Fig. 3. Sizes of blood spots on the Whatman 903 card depending on the hematocrit value of the applied whole blood sample: (A) Ht = 0.18, (B) Ht = 0.35, and (C) Ht = 0.50 [46].

of DBS technology in quantitative analysis. Hematocrit (Ht) is the volume ratio between red blood cells and a blood sample. The Ht value of a sample can significantly affect the accuracy of the determination of a target substance in DBS-based bioanalytical methods. Normal Ht values are about 0.41–0.51 and 0.37–0.47 for males and females, respectively [46]. However, these limits vary in certain population groups, for example, 0.28–0.67 in newborns (0–1 years) and 0.35–0.42 in children (2–12 years). The Ht level of capillary blood is usually higher than that in venous blood. The Ht value also varies in different species of animals and birds. The Ht value is directly proportional to blood viscosity, which affects the fluidity and diffusion properties of blood applied to a membrane (Fig. 3). At high Ht values of the test sample, the distribution of a blood drop over the membrane is difficult; in this case, the spot size on the carrier will be smaller than that of a droplet of the same volume but with a lower Ht value [47]. Obviously, the greater the whole blood volume per unit area of a dried spot, the greater the weight of the corresponding dried sample taken for analysis upon separating an equal part of the membrane; therefore, the concentrations of the identified analytes will vary.

The effect of hematocrit also depends on the physicochemical properties of the analyte and on the properties of the membrane material used. A relatively low Ht value leads to a negative deviation of the determined analyte concentrations (compared to an average Ht level of 0.45), while a positive deviation is observed at an increased Ht value. Differences in the distribution coefficients of analytes between blood cells and plasma lead to the fact that the hematocrit effect can manifest itself differently for different substances. Thus, Koster et al. [48] studied the relationship between Ht values and the degrees of extraction of substances from membranes based on the example of several immunosuppressants using five different DBS cards for sampling. The hematocrit effect appeared on all of the five cards. Depending on the type of cards, the results of the analysis varied significantly especially at extreme Ht values and elevated concentrations. At the elevated concentrations of the immunosuppressants tacrolimus, sirolimus, everolimus, ascomycin, and temsirolimus, the effect of hematocrit was much stronger than that at low concentrations. In general, the effect of hematocrit increases the scatter of the results of analysis to increase the total error, which can exceed an allowable value of 15% [49, 50].

Effect of the spot size. The application of different blood volumes to a membrane may cause different results in the determination of analyte concentration at a fixed size of the separated membrane section with the sample and a fixed Ht value of the sample. Denniff and Spooner [51] studied disks 3 mm in diameter from the spot center and perimeter and from the halo region around the spot. The halo effect occurs upon the drying of a blood spot, and it is characterized by a lighter shade of the edge than the main part of the spot. A significant (by more than 15%) deviation of the target analyte concentration from the values obtained from the central regions was observed in the halo region. This deviation is caused by an uneven analyte distribution and the sample-hematocrit effect. In the examinations of congenital diseases, the accuracy of determination of amino acids, acylcarnitines, and guanidinoacetate strongly depends on the spot area used in the analysis [51]. To overcome the effect of the spot size, a number of control reference samples of several concentrations and applied blood volumes are used. After drying, a membrane portion with the sample (for example, a disk with a diameter of 3 mm) is analyzed along with a set of calibration standards, for which the applied blood volume can be the same as one of the above or another. The difference in the results of LC–MS/MS analysis should not exceed 15% of the nominal value [20, 52, 53].

Chromatographic effect. When blood is applied to a paper membrane carrier, an uneven distribution of analytes may occur due to the chromatographic effect; as a result, the analyte concentrations at the center of the blood spot and on its periphery become different. Different results can be obtained depending on the type of cellulose membranes used [54, 55]. Previously, it was shown that target low-molecular-weight analytes can be unevenly distributed over the membrane component. Ren et al. [56] visualized the distribution of substances on various cellulose blood sampling cards (31 ETF, Grade 226, FTA Elute, and 903 FTA, Whatman, the United States) and demonstrated the uneven distributions of substances labeled with the isotope C^{14}. Moreover, the degree of uneven distribu-

tion of the target analyte on a particular membrane depended on the amount of applied blood in addition to the composition of the membrane. For example, for the FTA Elute card, a better result was obtained by applying smaller blood volumes (30 μL instead of 100 μL). It was also shown that the distribution of analytes on chemically modified cards, such as FTA and FTA Elute, was more dependent on external factors: air humidity and temperature significantly affected the accuracy of the analysis results. O'Mara et al. [57], who studied the distribution of glycogen synthases with different binding to plasma proteins, also observed an inhomogeneous distribution of the target analyte. Depending on the structure peculiarities of the test molecules, the chromatographic effect significantly influenced their distribution along the blood spot.

Introduction of an internal standard into a dried sample, the matrix effect. In the quantitative analysis of liquid samples by instrumental methods, the addition of an IS is simple, fast, and reliable, as compared to dried samples. The uniform distribution of an IS in a dried sample without preliminary elution is a difficult problem to be solved in MS detection, which is based on the direct passage of an eluting solution through a blood spot and the subsequent spraying [29]. In addition, the presence of a matrix can affect the accuracy of the results of MS determination. The use of solutions with high water content in combination with a polar organic solvent (methanol or acetonitrile) in DBS extraction can lead to the joint extraction of polar matrix components, which subsequently cause the effects of ion suppression or enhancement in MS analysis [2].

Ways to overcome the blood sample hematocrit effect. The effect of hematocrit is perhaps the most important obstacle to the widespread use of DBS technology in bioanalysis. Various approaches were currently proposed to overcome the effect of hematocrit, which were mainly oriented to taking a fixed sample volume. For example, the effect can be suppressed by limiting the absorbent surface area when the sample is applied to membrane areas in the form of disks of a certain size or perforated along the edge [35, 58–60]. Youhnovski et al. [58] used pre-cut cellulose disks fixed on adhesive tape. The entire pre-cut disk with the known volume of an applied blood sample is used for analysis. This approach reduces deviations in the analysis of samples with low and high hematocrit values, as compared to the average value (0.45). However, the results can still vary markedly for some analytes depending on the hematocrit values due to different analyte distributions between plasma and blood cells. Li et al. [59] also used pre-cut disks for the determination of acetaminophen. This approach made it possible to increase the accuracy of the analysis results. In addition, it has simplified the disposal of samples because the entire sample is taken for analysis without residue. Plastic cartridges or cases with an individual cellulose disk placed inside are other versions of pre-cut devices with disks of a certain diameter proposed in the literature [35]. Another way to overcome the hematocrit effect involves recalculating the found analyte concentrations based on the Ht values of particular samples. However, the Ht value should be determined for this purpose, and it is difficult to perform for dried samples although there are appropriate procedures. For example, the hematocrit can be determined with sufficient accuracy according to a well-known method based on the level of potassium in blood or by evaluating the reflection of ultraviolet radiation from the surface of a spot [61, 62], in particular, using an automatic system [63]. Other developments were focused on auxiliary devices that accurately applied the samples onto a membrane carrier. The effect of hematocrit can be eliminated if the entire blood spot is used in the analysis. Li et al. [64] presented a membrane filtration device for the separation of plasma from blood cell elements. The device consists of two different membranes: the first is a barrier to erythrocytes and it delays them, and the second serves as a carrier for the plasma separated from the cells. Thus, plasma samples can be obtained without prior centrifugation. This technology can become an alternative to DBS and other microsampling techniques (blood microsampling) because it completely eliminates the effect of hematocrit on the accuracy of dried sample analysis. A more radical way to overcome the disadvantages of the DBS analysis is the development of new alternative formats of dried spot devices including those containing membrane carriers of various compositions. Some devices are described in the next section.

DEVICES FOR DRIED SPOT COLLECTION ON MEMBRANE CARRIERS

Currently, cellulose cards are mainly used for the collection of dried biosamples; the production of these cards began with the successful application of the DBS technology in neonatal screening. Since then, the fundamental structure and composition of these cards have not undergone major changes. The card consists of an internal absorbent part, which is a cellulose material of a certain chemical and physical composition, and an external part with protective and informational functions (Fig. 2). On the inside, circles are outlined to indicate the places where biosample drops or tissue/organ imprint should be applied. Most cards for medical purposes are manufactured by Whatman (the United States), a large company that manufactures paper filters and related materials. Some other companies (Ahlstrom-Munktell in Sweden, Fisher Scientific in the United States, etc.) produce cards under their own brands, but they use a similar format and cellulose material (Whatman 903). This card format is convenient for applying capillary blood from a patient's finger, but a number of conditions, which are often vio-

Fig. 4. HemaSpot HF dried blood sampler (SpotOnScience, the United States).

lated, should be met for the correct application of blood. For example, a drop of blood should be applied to the center of a circle, while you cannot apply a drop in one circle twice. Deviations from the center often lead to the need to reapply a drop to the same area, and this causes a local increase in the substance concentration on the carrier and, as a result, an overestimation of the analysis results and a decrease in reproducibility. In addition, the cellulose material itself used as an absorbent membrane has a number of properties that make the subsequent analysis of a dried sample difficult. The effect of hematocrit and the chromatographic effect due to the physicochemical properties of hollow cellulose fibers are described above, and these effects interfere with the analysis. Moreover, the amount of adsorbed sample per unit area of the cellulose membrane is usually unknown. The use of cellulose material is currently rather a tribute to traditions and established market relations. Modern scientific developments are aimed not only at the development of new analytical approaches to DBS analysis but also at the use of more technologically advanced membrane materials. The role of these materials is not only to adsorb and preserve a biological sample but also to minimize the influence of various factors on the subsequent analysis of dried spots [65]. For example, the use of non-cellulosic materials for the production of DBSs has been described [66, 67]. It has been noted that non-cellulosic materials have better distribution parameters regardless of the viscosity of the applied liquid. However, the spot on these carriers can be uneven in shape, and the material is more fragile than paper when a disc is cut out [48].

To overcome the problems of sample preparation and analysis associated with the use of cellulose materials, many different devices have been proposed in order to obtain dried microsamples of a certain volume and facilitate the subsequent analysis [68]. A new dried spot collection device (HemaSpot TE from SpotOnScience, the United States) [69] is a case containing a porous cellulose carrier, made in the form of a propeller, inside (Fig. 4). A drop of blood is applied to the central part of the membrane; thereafter, it spreads along the petals of the membrane in the radial direction, the case is tightly closed, and the membrane is dried due to a desiccant inside. The petals of the membrane absorb a limited volume of blood; the effect of hematocrit decreases when they are completely filled. For the analysis, it is sufficient to separate one of the petals and desorb the analyte from the membrane. This approach to sample preparation, compared with a classical cellulose drop carrier (Munktel TFN), resulted in a higher correlation of results, as compared to rapid HIV tests in Nigeria [53]. This study showed that optimized procedures and strict adherence to the rules for using dried spot collection devices for sampling are required in underequipped laboratories and medical personnel. In addition, devices suitable for sampling in the field are required in order to obtain accurate analysis results and a high correlation with gold standard methods. The disadvantages of the HemaSpot system include its relative complexity in manufacturing and subsequent use because additional tools, such as dispensers, are required for applying blood samples. To achieve the high reproducibility of assay results, it is necessary to accurately apply an aliquot portion of blood to the center of the membrane and to control the amount of applied liquid so that it does not exceed the maximum capacity of the membrane. Moreover, if it is impossible to take a volume necessary for application (blood may flow poorly from the finger), the petals cannot be completely filled and hence the blood volume in the petal is unknown and the result of the analysis is incorrect. The manufacturer has also developed a Hemaspot SE device with a carrier in the form of a spiral: blood is applied to the center, and cell elements are separated in the process of spreading the sample over the carrier. Thus, it is possible to analyze a dried sample of blood or the corresponding plasma by separating a desired portion of the carrier.

Another volumetric absorptive microsampling (VAMS) device (Neoteryx, the United States) [70, 71] is a plastic rod with a small adsorption element made of a polymeric material in the form of a match head at the end (Fig. 5). According to Denniff and Spooner [70], the capacity of the element is fixed, and it can vary from 10 to 30 μL depending on the purpose of the device, as confirmed by introducing radioactive isotopes into a sample and measuring the radioactivity of the adsorbent with the sample. To apply blood, the end of the absorbent head is dipped into the sample

Fig. 5. Volumetric absorptive microsampling (VAMS) device (Neoteryx, the United States).

until complete saturation. The effect of hematocrit on the analysis in the range of Ht values 0.20–0.70 was insignificant, and the deviation from the conditionally normal value (0.45) was 5%, which is much lower than that for a classical cellulose membrane, where the measurement results varied within 30%.

Asante dried blood specimen collection strips (Sedia Biosciences, the United States) (Fig. 6) are plastic-based absorbent strips of the lateral type. Blood is absorbed into a sorption material about 2 cm long, which has a certain capacity for collecting a fixed volume of blood. Before analysis, the sorbed blood is completely extracted into solution. This reduces the effect of errors in blood sampling associated with an excess or insufficient amount of blood applied by the patient. However, dosing of a dried sample is not provided in this case; the applied and dried sample is completely washed off into the solution. A card from RayBiotech Life (the United States), which contains several strips for sampling about 30 μL of whole blood per strip, is based on a similar principle.

A HemaXis auxiliary device (DBS System SA, Switzerland) for taking a fixed volume of blood integrated with a standard card from Whatman was presented [72]. This device makes it possible to apply blood in a fixed volume to the membrane at the center of a circle using a capillary system (Fig. 7). This avoids errors in the application of blood by the patient, subject to subsequent extraction in automatic flow systems that use the entire area of the membrane with the applied dried sample.

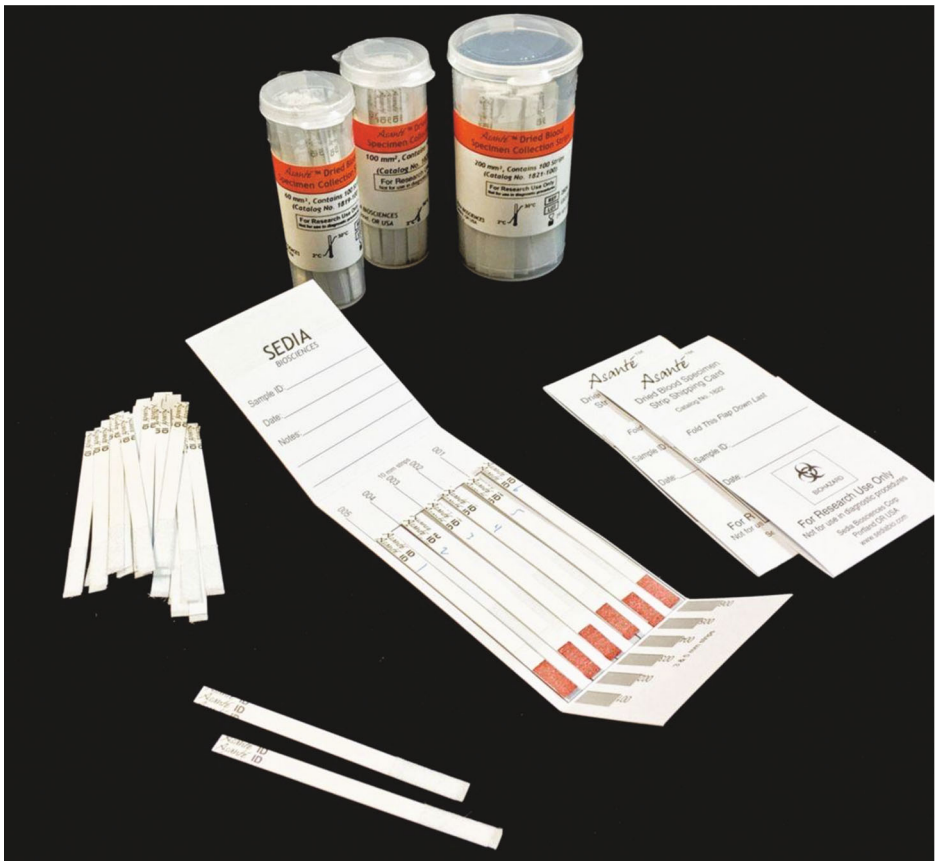

Fig. 6. Asante dried blood specimen collection strips (Sedia Biosciences Corporation, the United States).

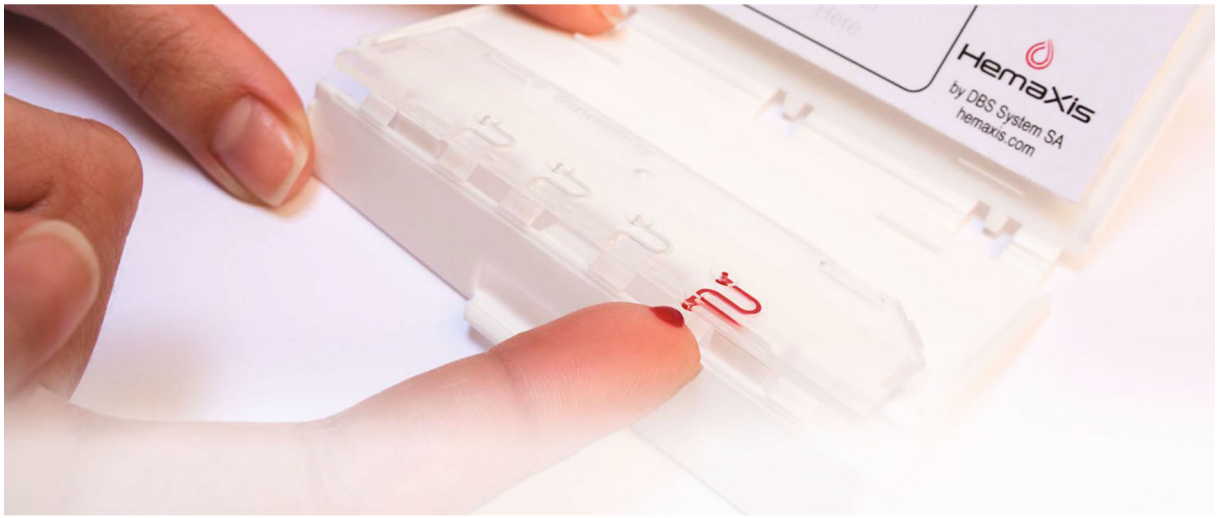

Fig. 7. HemaXis blood sampling system (DBS System SA, Switzerland).

Neto et al. [73] used a capillary for the accurate sampling of 3 μL of blood together with pre-cut disks of a cellulose material (Fig. 8). This system consists of membrane parts, onto which an exact volume of blood (3 μL) is applied from a capillary, fixed in a plastic cartridge. This system makes it possible to increase the accuracy of applying the samples in comparison with that of a semi-automatic sampler. However, undoubtedly, this complicates the device and sample application procedure. In terms of practicality, such devices are unlikely to compare with classic sampling cards, which are easy to manufacture, store, and transport.

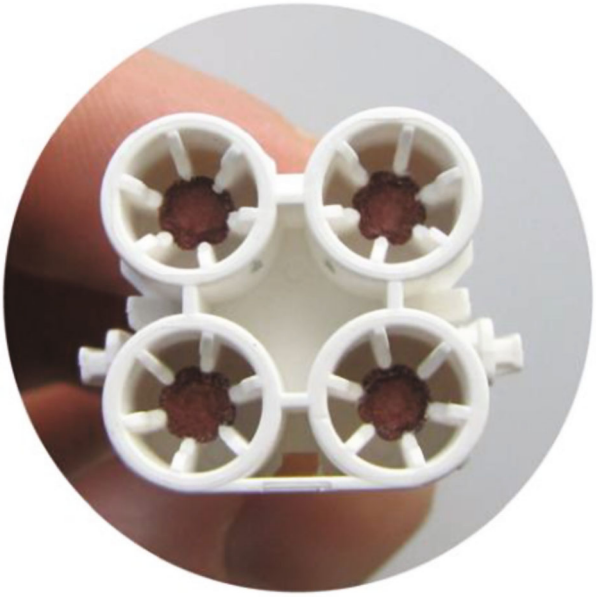

Fig. 8. Device for the capillary application of blood to pre-cut discs [73].

In addition to new devices, card formats and membrane materials that could serve as an alternative to the traditional rectangular card format were also described. For example, Polley et al. [74] used cellulose material strips with a broadened end to obtain DBSs (Fig. 9). According to Polley et al. [74], this format is convenient for sampling a certain volume of blood (about 20 μL) and the subsequent PCR analysis of blood for the diagnosis of malaria. Of course, this format is suitable for qualitative analysis, but it is not suitable for quantitative measurements because the sampling is inaccurate.

Nobuto [75] was the first to propose blood sampling on strips of cellulose material for the diagnosis of toxoplasmosis in 1966. The strips consisted of two parts: absorbent and distribution areas (Fig. 10). Subsequently, Nobuto strips were repeatedly used for veterinary diagnosis of various infectious diseases in both domestic and wild animals [31, 76–79].

Our scientific laboratory has been actively working on the preparation of biosamples in the form of dried spots over the past decade. The authors of this review proposed a new format for sample preparation of dried samples using narrow strips of highly porous material divided into zones of the same size (Fig. 11) [80, 81]. Such a strip of membrane material has two functions: first, the function of a biomaterial carrier for storing and transporting a dried sample to the laboratory and, second, the function of a microsampler, that is, a device capable of absorbing a fixed microamount of liquid as a capillary dispenser. The amount of sorbed liquid is determined by the sorption properties of the material used. This format allows one to dispense a dried sample using a required number of identical membrane sections in order to perform repeated studies or several types of analysis of a dried sample. In addition, the application of a sample using a strip is

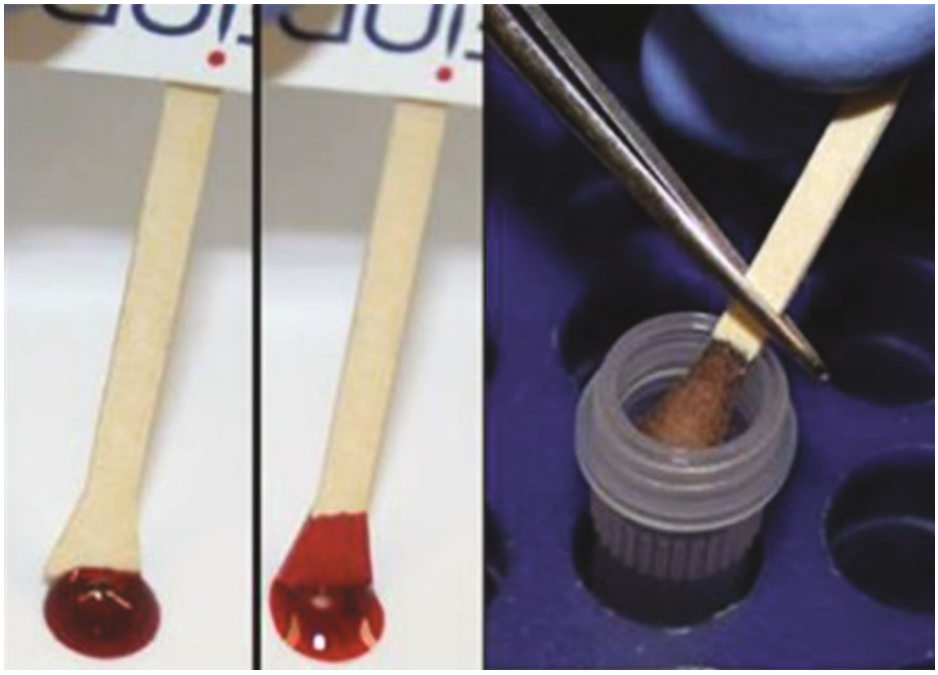

Fig. 9. Cards with a cellulose membrane carrier in the form of widened strips [74].

facilitated: the sample can be applied from the influx of blood at the puncture site or from a tube by dipping the edge of the strip into the liquid. In the former case, this greatly facilitates the collection of blood samples from animals and birds. A liquid, getting on the material, is easily absorbed, and it spreads along the strip of porous membrane material due to capillary forces. Numerous examples have shown that the distribution of both low-molecular-weight and high-molecular-weight substances along the strip occurs uniformly; because of this, any part of the membrane can be used for analysis without compromising the accuracy of the analysis [80–83]. It has also been shown that this condition is met for membrane materials made of whole glass fibers and polymer fibers, while a chromatographic effect occurs in cellulose material [80]. The study of the physicochemical properties of different membrane materials in terms of their applicability to this format and the subsequent use for dried sample preparation and analysis of biological fluids made it possible to recognize glass fiber material as the most effective for use in bioanalytics. For example, blood samples with different hematocrit values are distributed more evenly on glass fiber material than on cellulose material (Fig. 12). In this case, the distribution uniformity depends on the sorption properties of the membrane. The successful use of the proposed sample preparation format in combination with quantitative, semi-quantitative, and qualitative immunochemical and PCR analysis was demonstrated using the detection of hormones, protein metabolites, antibodies, and DNA in the samples of whole blood, serum, plasma and whole milk for the purposes of biomedical and veterinary diagnostics as examples [80–93].

APPLICATION OF THE DRIED SPOT TECHNOLOGY FOR THE SAMPLING OF BIOMATERIALS ON POROUS CARRIERS

Currently, the sample preparation of biomaterials in the form of dried spots is mainly used to determine low-molecular-weight substances, such as biologically active pharmaceutical compounds and illegal drugs [7, 8, 11, 18], and to collect biomaterials for PCR and genetic studies [22, 30, 42, 43, 94]. The DBS method is well suited for therapeutic drug monitoring, which requires multiple measurements during therapy with substances with short exposure intervals [44]. The DBS technology occupies a special place in the remote diagnostics of human diseases and in neonatal screening [43].

Monitoring of human diseases. According to McDade et al. [95], at least 45 analytes, the identification of which in DBS analysis is of the greatest value for population studies, can be recognized. Among

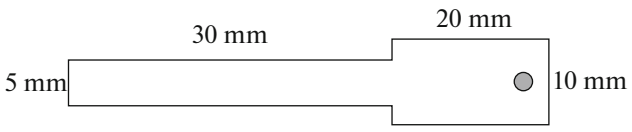

Fig. 10. Schematic diagram of a Nobuto cellulose strip [75].

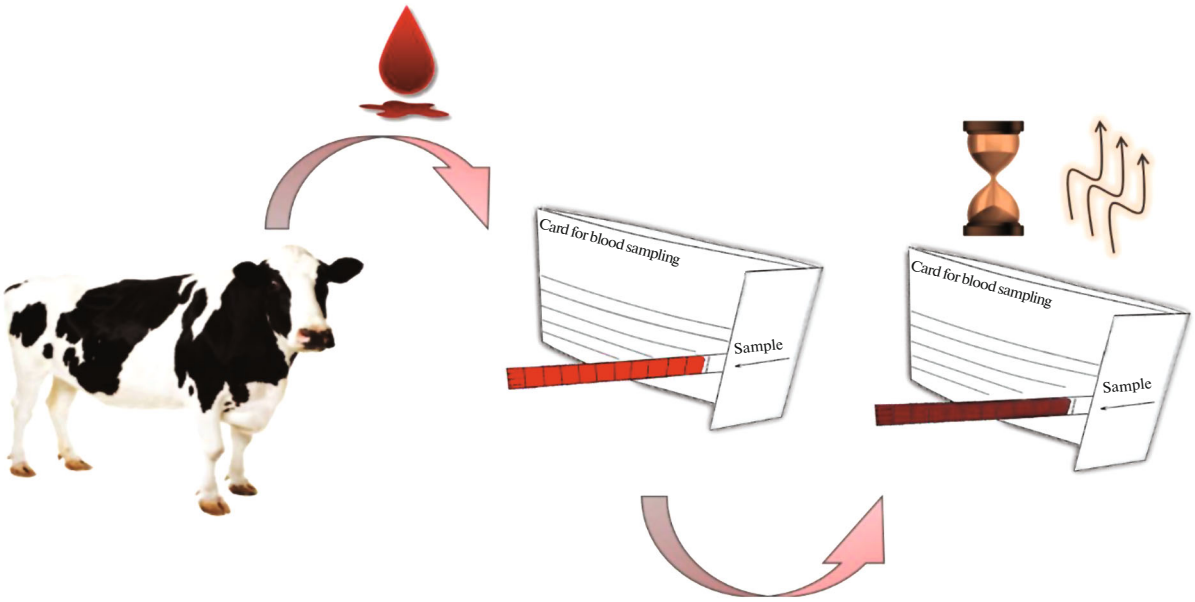

Fig. 11. New format for the preparation of dried samples on a marked narrow strip of membrane material.

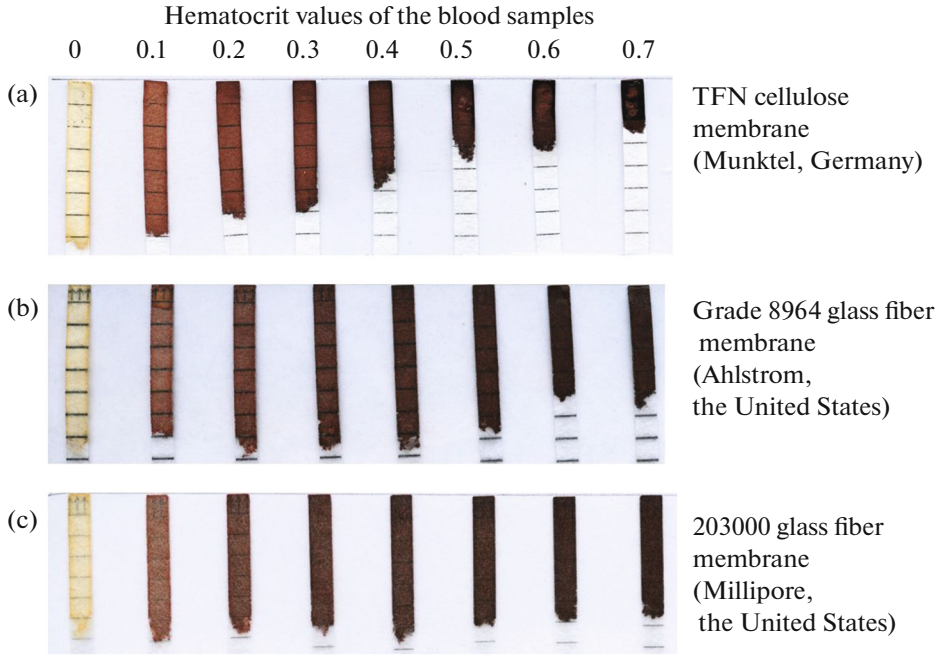

Fig. 12. Spread of 80 μL of blood along a narrow strip of membrane material depending on the hematocrit values of the blood samples.

them are biomarkers that characterize the state of human endocrine, cardiovascular, reproductive, and immune systems, which can be detected in a small volume of the test sample at the current level of the development of diagnostic technologies. The DBS technology is now being integrated into several national US and international population-based research programs for older children, adults, and the elderly. Thus, the DBS technology has found wide application in the remote studies of hepatitis [96] and HIV in African countries [12, 13, 15]. Efforts to increase the availability of HIV testing in rural areas of developing countries, where more than 90% of all people with HIV live, are critical to fighting the disease. The DBS tech-

nology provides a simple, reliable, and affordable way to collect blood and other body fluids for disease screening, quality control of health care, measurement of HIV viral load, and drug resistance testing under conditions when venous blood sampling and transport are not feasible or difficult to perform [14]. Most quantitative HIV viral load tests are performed by the PCR method, which requires a large amount of plasma (100–600 μL) and the transcription of RNA to DNA prior to amplification. In addition to the extracellular HIV-1 RNA found in plasma samples, dried samples contain whole blood and, therefore, intracellular HIV-1 RNA and HIV-1 proviral DNA. As a result, viral load testing using the DBS technology is potentially more sensitive than the determination of HIV-1 in plasma or serum. This is of great importance for the early detection of HIV [21].

Preclinical and clinical drug studies. Preclinical trials are based on the sampling of blood from experimental animals within a certain period of time after the administration of a drug in order to determine the exact concentrations of the substances in the blood. Preclinical trials include the study of the pharmacological and toxicological properties of drugs. Currently, many studies and publications are devoted to this particular area of using the DBS technology in pharmacokinetics [4, 27, 49, 50, 97, 98]. A standard procedure for performing analyzes and pharmacokinetic studies involves the serial sampling of a sufficiently large volume of blood (usually about 1 mL) from animals (rats or mice), while the animal dies in the course of blood sampling. A change to the DBS technology can significantly reduce the amount of taken biological material and save significant funds. In addition, the same animal can be repeatedly used in a series of DBS measurements [98].

Therapeutic drug monitoring. Due to low injury rate, the DBS technology is indispensable in the therapeutic monitoring of drugs when is necessary to monitor the dynamics of drug concentration in the body in order to prescribe a required course of administration. This is especially true for drugs with a short therapeutic time of exposure or for unstable drugs. For example, Marca et al. [99] used LC–MS/MS to analyze DBSs for the presence of immunosuppressors. The DBS technology, especially in combination with LC–MS/MS analysis, is actively used in the clinical trials of antipyretic, antitussive, antiviral, anticonvulsant, immunosuppressive, antiepileptic, and other drugs. However, with the use of the DBS technology in therapeutic monitoring, it is necessary to take into account factors affecting the results of the analysis of dried and liquid samples (for example, hematocrit) [95].

Neonatal screening. The use of the DBS technology in neonatal screening has become widespread throughout the world [4]. Due to the small volume of blood taken, the traumatization of newborns is reduced, blood sampling is facilitated, and early diagnosis of pathologies becomes possible. The etiology and pathogenesis of congenital diseases are mainly revealed in infants. Such diseases are very rare, and they amount to units per thousand and tens of thousands of newborns; however, their timely diagnosis and follow-up guarantee that the child will be free from the negative manifestations of the disease. For example, when phenylketonuria (phenylalanine metabolism disorders) is detected, a special diet that excludes the consumption of high-protein foods is prescribed to the child. The limitation of phenylalanine consumption by the body makes it possible to avoid irreversible pathological changes leading to mental retardation [100].

Dried blood spot technology in veterinary medicine and biology. Despite the fact that the DBS technology is relatively widely used in medical research, its significance for veterinary medicine and biology is clearly underestimated today. In fact, the use of the DBS technology for the collection, storage, and analysis of dried samples in the diagnosis of diseases of farm animals, poultry, domestic, and wild animals is only at the very initial stage of development, although successful attempts to study dried blood samples of farm animals obtained on membrane materials using serological methods have been described as early as the 1950s–1960s [74, 101]. In the subsequent several years, the number of scientific works not only in medicine but also in veterinary medicine sharply increased. Over the next 30 years, the technology did not arouse serious interest in veterinary medicine and biology, and the number of publications began to grow only in the late 1990s and early 2000s following the widespread use of the DBS technology in the world. However, even the current total number of publications devoted to the use of DBS in veterinary diagnostics and biological research is small, and it amounts to about 250 works.

Veterinary diagnostics based on the DBS technology is carried out using a wide range of methods, mainly including serodiagnostics to detect specific antibodies in the blood of an animal and PCR to detect the pathogen genome. Instrumental methods of analysis, such as LC, MS, etc., are used to determine antibiotics, pesticides, and metals [102–107]. The use of samples dried on a carrier is of great practical interest for the monitoring of diseases in regions with a tropical climate, hard-to-reach and remote places, and countries with a vast territory, where there may be difficulties in transporting and storing the samples. The DBS technology has been repeatedly used to monitor important infectious diseases in agricultural and domestic animals, such as trypanosomal diseases of livestock in African countries [108], and to study vector organisms involved in the transmission of infection [109]. An interesting direction of such research is the analysis of mosquito saliva and the parts of vector insects, such as tsetse flies, ticks, etc., in

order to identify the pathogen genome [110, 111]. Hopkins et al. [112] performed the identification of infected and non-infected animals by a serological method based on the results obtained using the DBS technology, which was more efficient than the analysis of liquid samples. It was noted that much more effort is required to obtain serum for the standard analysis of a liquid sample in the field because a significant amount of material should be collected from the tail or jugular vein of cattle.

The DBS technology finds application in the monitoring of diseases of various wild animals and birds [21, 30, 31, 76–78, 113–115]. Most infectious diseases in wildlife are poorly controlled. Due to the impossibility of taking many samples in a day, the samples are accumulated over a long period of time. The DBS technology makes it possible to store dried samples during the entire expedition without refrigeration. This approach was used in the monitoring of reindeer brucellosis in Canada [77]. This disease is difficult to control due to the harsh climate and the vast habitat of wild animals. Livestock are also susceptible to the disease and the main cause of infection is transmission from wild animals. Moreover, the danger of human infection cannot be ruled out with the insufficient heat treatment of food. Samples were taken from deer killed in the course of shooting under extreme conditions at temperatures down to –40°C using Nobuto strips and analyzed by ELISA two months later. The collection of dried samples on membrane carriers is used in genetic and morphological studies, for example, in order to determine the genome and serotype of the pathogens of infectious diseases and to create biobanks [22, 43]. The anthropogenic impact on the representatives of wild fauna is studied with the use of the DBS technology. For this purpose, the presence of toxicants, heavy metals, pesticides, and antibiotics in the blood of migratory birds is determined [102–106].

It is promising to use the DBS technology for the monitoring of infectious diseases in farm animals and the level of post-vaccination immunity [85, 91]. Intensively managed farm animals and poultry are susceptible to a large number of infectious diseases, usually, of a viral nature, and the infection can spread quickly and affect a large number of animals under industrial conditions. There are special livestock vaccination programs for the prevention of infectious diseases depending on the prevalence in a particular region. Most publications on the use of the DBS technology noted that the successful fight against dangerous infections in wild and farm animals requires the development of an effective and inexpensive method for storing and transporting blood samples. This method is the collection of the dried spots of biological fluids on membrane carriers [22, 43, 76, 79, 94]. However, this technology is unpopular and little known in the veterinary practice of Russia; there are no approved methodological guidelines for the use of dried samples in monitoring programs. For animals, the possibilities of applying blood and getting dried spots with the use of classic cards are limited. Note that, in some studies, the researchers themselves cut the cellulose material into strips for the convenience of applying the samples in order to impregnate the membrane directly from the puncture and conveniently measure at the subsequent stages [38, 116, 117].

PROSPECTS FOR THE DEVELOPMENT OF THE DRIED BLOOD SPOT TECHNOLOGY

The method of blood microsampling on a membrane carrier will be increasingly used in the development of new drugs, where it is necessary to regularly take the samples of biological fluids from laboratory animals. It is obvious that the DBS technology will also be extended to methods for collecting and analyzing the samples of not only whole blood but also other biological fluids of humans and animals [2, 15, 83, 84]. At this stage of the development, many operations in sample preparation are carried out manually. Although this is not a problem with small samples, large-scale research requires the use of automated technologies. Currently available devices make it possible to fully automate the processing of cards with dried spot samples for the subsequent automated analysis [23, 63]. In parallel with a decrease in the amount of sampled material and the miniaturization of liquid microsampling devices, the analyzers that simultaneously include DBS sample preparation units and a mass spectrometer are miniaturized. Attempts are being made to interface automated DBS devices with instruments used in other methods in order to expand the range of test materials and increase the accuracy of the analysis. The development of DBS methods with the use of LC–MS/MS analysis, which significantly expands the capabilities of the technology due to a combination of several techniques that mutually compensate the disadvantages of each other, was reported [2]. With strict adherence to a sampling procedure, the automated separation of the membrane section with the sample and the extraction (elution) of analytes, and an improvement of the chemical composition of sorbing porous membranes to increase the stability of unstable compounds, the DBS technology will play an important role in the quantitative analysis of drugs, the determination of metabolites in blood, and the diagnosis of diseases in medicine and veterinary medicine [15]. The range of compounds to be determined with the use of the DBS is technology expanding; methods for the determination of trace elements [117] and saturated and polyunsaturated fatty acids [118] are being developed. A new direction for the use of the DBS technology was announced in July 2021. At the 138th Session of the International Olympic Committee (IOC), the World Anti-Doping Agency (WADA) announced the approval of a method for testing dried blood spots of athletes for prohibited (doping) substances in international competitions, including the

Olympic Games, starting with the Tokyo 2021 Olympics.

* * *

In summary, note that, despite the wide distribution, porous cellulose carriers have generally recognized disadvantages: the uneven application and distribution of blood spots, the dependence of the area of a blood spot distribution zone on the viscosity properties of individual samples, and the incomplete washing of the sample from the carrier due to the partially irreversible sorption of blood components on cellulose fibers. These disadvantages limit the capabilities of the DBS technology used in quantitative analysis for diagnostic purposes. This is especially true for immunochemical methods, although the technology for neonatal screening is well developed and standardized. New porous non-cellulose membrane materials that have undeniable operational and analytical advantages and decrease or completely eliminate undesirable effects inherent in cellulose are currently proposed in order to obtain the dried spots of biosamples. In addition, more convenient formats of biosampling devices are being searched for, which will allow efficient sampling not only from humans but also from animals, in particular, outside the laboratory. It should be noted that most of the new devices for sampling and sample preparation in the form of dried spots are designed for individual use rather than for routine large-scale screening. The cost of an improved personal blood sampling device and the complexity of its manufacture and use suggest its applications in medicine. The use of biosamples in the form of dried spots for the monitoring of infectious diseases is not widespread in veterinary diagnostics. In particular, this is due to the high cost of sampling devices for medical use available on the market and the absence of alternative sample preparation systems and formats convenient for veterinary use, in particular, for taking blood samples from animals and birds in the field. In addition, methodological approaches to working with dried samples are poorly developed. Through the development of convenient sampling devices, the DBS technology can become an alternative to the use of liquid samples for the routine monitoring of farm animals and poultry and other veterinary and biological studies of wildlife. The use of medical devices based on cellulose membranes, mainly in the form of cards, in diagnostics is focused on quantitative analysis performed by physicochemical methods in order to assess the level of low-molecular-weight substances in human blood. The duration of the analysis and the list of analytes strongly depend on the possibility of automating the stages of operation with dried samples. The availability of automatic sample preparation stations is a serious barrier to the widespread introduction of the technology in practice in developing countries. The use of dried samples creates additional methodological difficulties for routine immunochemical analysis in laboratories focused on the analysis of liquid samples because most operations with DBSs are performed manually. Despite its growing popularity, the DBS technology is not yet fully established in some analytical aspects. In general, the DBS technology is evolving toward improvements and increasing the fraction of automated processes, which will ultimately lead to a reduction in the average analysis time and to the possibility of performing large-scale research and disease monitoring. The accuracy and sensitivity of the results obtained using the dried spots of biological fluids increases with the development of modern technologies. Obviously, the use of the DBS technology will gradually replace traditional liquid plasma analysis. The indisputable advantages of the DBS technology are the simplicity of blood sampling by the patient himself, the possibility of sending a dried sample by mail and obtaining the result of the analysis remotely, and the possibility of delivering a dried sample to the laboratory from remote areas, for example, from African countries. This delivery method can simplify the work and contribute to the detection and eradication of diseases such as HIV and hepatitis B in third world countries.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Guthrie, R. and Ada, S., *Pediatrics*, 1963, vol. 32, no. 3, p. 338.
2. Meesters, R.J. and Hooff, G.P., *Bioanalysis*, 2013, vol. 5, no. 17, p. 2187. https://doi.org/10.4155/bio.13.175
3. Demirev, P.A., *Anal. Chem.*, 2013, vol. 85, no. 2, p. 779. https://doi.org/10.1021/ac303205m
4. Sharma, A., Jaiswal, S., Shukla, M., and Lal, J., *Drug Test. Anal.*, 2014, vol. 6, no. 5, p. 399. https://doi.org/10.1002/dta.1646
5. Diener, C., Galata, V., Keller, A., and Meese, E., *Crit. Rev. Clin. Lab. Sci.*, 2019, vol. 56, no. 2, p. 111. https://doi.org/10.1080/10408363.2018.1561641
6. Antunes, M.V., Charão, M.F., and Linden, R., *Clin. Biochem.*, 2016, vol. 49, nos. 13–14, p. 1035. https://doi.org/10.1016/j.clinbiochem.2016.05.004
7. Lim, M.D., *Am. J. Trop. Med. Hyg.*, 2018, vol. 99, no. 2, p. 256. https://doi.org/10.4269/ajtmh.17-0889
8. Stove, C.P., Ingels, A.S., De Kesel, P.M., and Lambert, W.E., *Crit. Rev. Toxicol.*, 2012, vol. 42, no. 3, p. 230. https://doi.org/10.3109/10408444.2011.650790
9. Enderle, Y., Foerster, K., and Burhenne, J., *J. Pharm. Biomed. Anal.*, 2016, vol. 130, p. 231. https://doi.org/10.1016/j.jpba.2016.06.026

10. Malsagova, K., Kopylov, A., Stepanov, A., Butkova, T., Izotov, A., and Kaysheva, A., *Diagnostics*, 2020, vol. 10, no. 3, p. 248. https://doi.org/10.3390/diagnostics10040248
11. McClendon-Weary, B., Putnick, D.L., Robinson, S., and Yeung, E., *Curr. Environ. Health Rep.*, 2020, vol. 7, p. 211. https://doi.org/10.1007/s40572-020-00289-y
12. Smit, P.W., Sollis, K.A., Fiscus, S., Ford, N., Vitoria, M., Essajee, S., Barnett, D., Cheng, B., Crowe, S.M., Denny, T., Landay, A., Stevens, W., Habiyambere, V., Perriens, J.H., and Peeling, R.W., *PLoS One*, 2014, vol. 9, no. 3, e86461. https://doi.org/10.1371/journal.pone.0086461
13. Parry, C.M., Parkin, N., Diallo, K., Mwebaza, S., Batamwita, R., DeVos, J., Bbosa, N., Lyagoba, F., Magambo, B., Jordan, M.R., Downing, R., Zhang, G., Kaleebu, P., Yang, C., and Bertagnolio, S., *J. Clin. Microbiol.*, 2014, vol. 52, no. 8, p. 2868. doi https://doi.org/10.1128/jcm.00544-14
14. Solomon, S.S., Solomon, S., Rodriguez, I.I., McGarvey, S.T., Ganesh, A.K., Thyagarajan, S.P., Mahajan, A.P., and Mayer, K.H., *Int. J. STD AIDS*, 2002, vol. 13, no. 1, p. 25. https://doi.org/10.1258/0956462021924578
15. *Dried Blood Spots: Applications and Techniques*, Li, W. and Lee, M.S., Eds., New York: Wiley, 2014, p. 363.
16. Lehmann, S., Picas, A., Tiers, L., Vialaret, J., and Hirtz, C., *Crit. Rev. Clin. Lab. Sci.*, 2017, vol. 54, no. 3, p. 173. https://doi.org/10.1080/10408363.2017.1297358
17. Freeman, J.D., Rosman, L.M., Ratcliff, J.D., Strickland, P.T., Graham, D.R., and Silbergeld, E.K., *Clin. Chem.*, 2018, vol. 64, no. 4, p. 656. https://doi.org/10.1373/clinchem.2017.275966
18. Li, W. and Tse, F.L.S., *Biomed. Chromatogr.*, 2010, vol. 24, no. 1, p. 49. https://doi.org/10.1002/bmc.1367
19. Wagner, M., Tonoli, D., Varesio, E., and Hopfgartner, G., *Mass Spectrom. Rev.*, 2016, vol. 35, no. 3, p. 361. https://doi.org/10.1002/mas.21441
20. Jager, N.G., Rosing, H., Schellens, J.H., and Beijnen, J.H., *Bioanalysis*, 2014, vol. 6, no. 18, p. 2481. https://doi.org/10.4155/bio.14.185
21. Smit, P.W., Elliott, I., Peeling, R.W., Mabey, D., and Newton, P.N., *Am. J. Trop. Med. Hyg.*, 2014, vol. 90, no. 2, p. 195. https://doi.org/10.4269/ajtmh.13-0463
22. Smith, L.M. and Burgoyne, L.A., *BMC Ecol.*, 2004, vol. 4, no. 4. https://doi.org/10.1186/1472-6785-4-4
23. Luginbuhl, M. and Gaugler, S., *Clin. Biochem.*, 2020, vol. 82, p. 33. https://doi.org/10.1016/j.clinbiochem.2020.02.007
24. Thomas, A., Déglon, J., Steimer, T., Mangin, P., Daali, Y., and Staub, C., *J. Sep. Sci.*, 2010, vol. 33, nos. 6–7, p. 873. https://doi.org/10.1002/jssc.200900593
25. Wong, P., Pham, R., Whitely, C., Soto, M., Salyers, K., James, C., and Bruenner, B.A., *J. Pharm. Biomed. Anal.*, 2011, vol. 56, no. 3, p. 604. https://doi.org/10.1016/j.jpba.2011.06.022
26. Déglon, J., Thomas, A., Mangin, P., and Staub, C., *Anal. Bioanal. Chem.*, 2012, vol. 402, no. 8, p. 2485. https://doi.org/10.1007/s00216-011-5161-6
27. Emmons, G. and Rowland, M., *Bioanalysis*, 2010, vol. 2, no. 11, p. 1791. https://doi.org/10.4155/bio.10.159
28. Abu-Rabie, P., Denniff, P., Spooner, N., Brynjolffssen, J., Galluzzo, P., and Sanders, G., Anal. Chem., 2011, vol. 83, no. 22, p. 8779. https://doi.org/10.1021/ac202321q
29. Meesters, R., Hooff, G., van Huizen, N., Gruters, R., and Luider, T., *Bioanalysis*, 2011, vol. 3, no. 20, p. 2357. https://doi.org/10.4155/bio.11.202
30. Cardona-Ospina, J.A., Villalba-Miranda, M.F., Palechor-Ocampo, L.A., Mancilla, L.I., and Sepulveda-Arias, J.C., *Prev. Vet. Med.*, 2019, vol. 172, 104772. https://doi.org/10.1016/j.prevetmed.2019.104772
31. Curry, P.S., Ribble, C., Sears, W.C., Orsel, K., Hutchins, W., Godson, D., Lindsay, R., Dibernardo, A., Campbell, M., and Kutz, S.J., *J. Wildl. Dis.*, 2014, vol. 50, no. 2, p. 308. https://doi.org/10.7589/2012-06-150
32. van Berkel, G.J., Pasilis, S.P., and Ovchinnikova, O., *J. Mass Spectrom.*, 2008, vol. 43, no. 9, p. 1161. https://doi.org/10.1002/jms.1440
33. Chace, D.H., *J. Mass Spectrom.*, 2009, vol. 44, no. 2, p. 163. https://doi.org/10.1002/jms.1528
34. Hernandes, V.V., Barbas, C., and Dudzik, D., *Electrophoresis*, 2017, vol. 18, p. 2232. https://doi.org/10.1002/elps.201700086
35. Meesters, R.J., Zhang, J., van Huizen, N.A., Hooff, G.P., Gruters, R.A., and Luider, T.M., *Bioanalysis*, 2012, vol. 4, no. 16, p. 2027. https://doi.org/10.4155/bio.12.175
36. Meesters, R.J., Hooff, G.P., Gruters, R., van Kampen, J.J., and Luider, T.M., *Bioanalysis*, 2012, vol. 4, no. 3, p. 237. https://doi.org/10.4155/bio.11.328
37. Kehler, J.R., Bowen, C.L., Boram, S.L., and Evans, C.A., *Bioanalysis*, 2010, vol. 2, no. 8, p. 1461. https://doi.org/10.4155/bio.10.108
38. Randriamparany, T., Kouako, K.V., Michaud, V., Fernández-Pinero, J., Gallardo, C., le Potier, M.F., Rabenarivahiny, R., Couacy-Hymann, E., Raherimandimby, M., and Albina, E., *Transboundary Emerging Dis.*, 2016, vol. 63, no. 4, p. 379. https://doi.org/10.1111/tbed.12295
39. Sharma, R., Parker, S., Al-Adhami, B., Bachand, N., and Jenkins, E., *Food Waterborne Parasitol.*, 2019, vol. 15, e00046. https://doi.org/10.1016/j.fawpar.2019.e00046
40. Strnadová, K.A., Holub, M., Mühl, A., Heinze, G., Ratschmann, R., Mascher, H., Stöckler-Ipsiroglu, S., Waldhauser, F., Votava, F., Lebl, J., and Bodamer, O.A., *Clin. Chem.*, 2007, vol. 53, no. 4, p. 717. https://doi.org/10.1373/clinchem.2006.076679
41. Fingerhut, R., Ensenauer, R., Róschinger, W., Arnecke, R., Olgemöller, B., and Roscher, A.A., *Anal.*

Chem., 2009, vol. 81, no. 9, p. 3571. https://doi.org/10.1021/ac8022235

42. Michaud, V., Gil, P., Kwiatek, O., Prome, S., Dixon, L., Romero, L., le Potier, M.F., Arias, M., Couacy-Hymann, E., Roger, F., Libeau, G., and Albina, E., *J. Virol. Methods*, 2007, vol. 146, nos. 1–2, p. 257. https://doi.org/10.1016/j.jviromet.2007.07.006
43. Madhanmohan, M., Yuvaraj, S., Manikumar, K., Kumar, R., Nagendrakumar, S.B., Rana, S.K., and Srinivasan, V.A., *Transboundary Emerging Dis.*, 2016, vol. 63, p. 675. https://doi.org/10.1111/ tbed.12316
44. Alfazil, A.A. and Anderson, R.A., *J. Anal. Toxicol.*, 2008, vol. 32, no. 7, p. 511. https://doi.org/10.1093/jat/32.7.511
45. Prieto, J.A., Andrade, F., Lage, S., and Aldámiz-Echevarría, L., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2011, vol. 879, nos. 11–12, p. 671. https://doi.org/10.1016/j.jchromb.2011.01.031
46. De Kesel, P.M., Sadones, N., Capiau, S., Lambert, W.E., and Stove, C.P., *Bioanalysis*, 2013, vol. 5, no. 16, p. 2023. https://doi.org/10.4155/bio.13.156
47. Adam, B.W., Alexander, J.R., Smith, S.J., Chace, D.H., Loeber, J.G., Elvers, L.H., and Hannon, W.H., *Clin. Chem.*, 2000, vol. 46, no. 1, p. 126. https://doi.org/10.1093/clinchem/46.1.126
48. Koster, R.A., Botma, R., Greijdanus, B., Uges, D.R.A., Kosterink, J.G.W., Touw, D.J., and Alffenaar, J.W.C., *Bioanalysis*, 2015, vol. 7, no. 10, p. 1225. https://doi.org/10.4155/bio.15.63
49. Beaudette, P. and Bateman, K.P., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2004, no. 809, p. 153. https://doi.org/10.21273/HORTTECH.25.4.528
50. Pandya, H.C., Spooner, N., and Mulla, H., *Bioanalysis*, 2011, vol. 3, no. 7, p. 779. https://doi.org/10.4155/bio.11.19
51. Denniff, P. and Spooner, N., *Bioanalysis*, 2010, vol. 2, no. 8, p. 1385. https://doi.org/10.4155/bio.10.103
52. Spooner, N., Lad, R., and Barfield, M., *Anal. Chem.*, 2009, vol. 81, no. 4, p. 1557. https://doi.org/10.1021/ac8022839
53. Liang, X., Li, Y., Barfield, M., and Ji, Q.C., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2009, vol. 877, nos. 8–9, p. 799. https://doi.org/10.1016/j.jchromb.2009.02.015
54. Holub, M., Tuschl, K., Ratschmann, R., Strnadová, K.A., Mühl, A., Heinze, G., Sperl, W., and Bodamer, O.A., *Clin. Chim. Acta*, 2006, vol. 373, nos. 1–2, p. 27. https://doi.org/10.1016/j.cca.2006.04.013
55. Zheng, N., Yuan, L., Ji, Q.C., Mangus, H., Song, Y., Frost, C., Zeng, J., Aubry, A.F., and Arnold, M.E., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2015, vol. 988, p. 66. https://doi.org/10.1016/j.jchromb.2015.02.023
56. Ren, X., Paehler, T., Zimmer, M., Guo, Z., Zane, P., and Emmons, G.T., *Bioanalysis*, 2010, vol. 2, no. 8, p. 1469. https://doi.org/10.4155/bio.10.96
57. O'Mara, M., Hudson-Curtis, B., Olson, K., Yueh, Y., Dunn, J., and Spooner, N., *Bioanalysis*, 2011, vol. 3, no. 20, p. 2335. https://doi.org/10.4155/bio.11.220
58. Youhnovski, N., Bergeron, A., Furtado, M., and Garofolo, F., *Rapid Commun. Mass Spectrom.*, 2011, vol. 25, no. 19, p. 2951. https://doi.org/10.1002/rcm.5182
59. Li, F., Zulkoski, J., Fast, D., and Michael, S., *Bioanalysis*, 2011, vol. 3, no. 20, p. 2321. https://doi.org/10.4155/bio.11.219
60. Li, F., Ploch, S., Fast, D., and Michael, S., *J. Mass Spectrom.*, 2012, vol. 47, no. 5, p. 655. https://doi.org/10.1002/jms.3015
61. Capiau, S., Stove, V.V., Lambert, W.E., and Stove, C.P., *Anal. Chem.*, 2013, vol. 85, no. 1, p. 404. https://doi.org/10.1021/ac303014b
62. Miller, J.H. IV, Poston, P.A., Rutan, S.C., and Thomas, K.H., *J. Anal. Bioanal. Tech.*, 2013, vol. 4, no. 1, p. 1. https://doi.org/10.4172/2155-9872.1000162
63. Luginbühl, M., Fischer, Y., and Gaugler, S., *J. Anal. Toxicol.*, 2020, bkaa189. https://doi.org/10.1093/jat/bkaa189
64. Li, Y., Henion, J., Abbott, R., and Wang, P., *Rapid Commun. Mass Spectrom.*, 2012, vol. 26, no. 10, p. 1208. https://doi.org/10.1002/rcm.6212
65. Velghe, S., Delahaye, L., and Stove, C.P., *J. Pharm. Biomed. Anal.*, 2019, vol. 163, p. 188. https://doi.org/10.1016/j.jpba.2018.10.010
66. Li, W., Doherty, J.P., Kulmatycki, K., Smith, H.T., and Tse, F.L., *Bioanalysis*, 2012, vol. 4, no. 12, p. 1429. https://doi.org/10.4155/bio.12.119
67. Lawson, G., Cocks, E., and Tanna, S., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2012, vol. 897, p. 72. https://doi.org/10.1016/j.jchromb.2012.04.013
68. Delahaye, L., Veenhof, H., Koch, B.C.P., Alffenaar, J.C., Linden, R., and Stove, C., *Ther. Drug Monit.*, 2021, vol. 43, no. 3, p. 310. https://doi.org/10.1097/FTD.0000000000000864
69. Rosypal, A.C., Pick, L.D., Hernandez, J.O.E., and Lindsay, D.S., *Vet. Parasitol.*, 2014, vol. 205, nos. 1–2, p. 338. https://doi.org/10.1016/j.vetpar.2014.07.031
70. Denniff, P. and Spooner, N., *Anal. Chem.*, 2014, vol. 86, no. 16, p. 8489. https://doi.org/10.1021/ac5022562
71. Kip, A.E., Kiers, K.C., Rosing, H., Schellens, J.H.M., Beijnen, J.H., and Dorlo, T.P.C., *J. Pharm. Biomed. Anal.*, 2017, vol. 135, p. 160. https://doi.org/10.1016/j.jpba.2016.12.012
72. Leuthold, L.A., Heudi, O., Déglon, J., Raccuglia, M., Augsburger, M., Picard, F., Kretz, O., and Thomas, A., *Anal. Chem.*, 2015, vol. 8, no. 4, p. 2068. https://doi.org/10.1021/ac503931g
73. Neto, R., Gooley, A., Breadmore, M.C., Hilder, E.F., and Lapierre, F., *Anal. Bioanal. Chem.*, 2018, vol. 410, no. 14, p. 3315. https://doi.org/10.1007/s00216-018-0993-y
74. Polley, S.D., Bell, D., Oliver, J., Tully, F., Perkins, M.D., Chiodini, P.L., and Gonzalez, I.J.,

Malar. J., 2015, vol. 14, no. 1, p. 1. https://doi.org/10.1186/s12936-015-0558-x

75. Nobuto, K., *Jpn. Agric. Res. Q.*, 1966, vol. 1, no. 3, p. 11.
76. Curry, P.S., Ribble, C., Sears, W.C., Hutchins, W., Orsel, K., Godson, D., Lindsay, R., Dibernardo, A., and Kutz, S.J., *J. Wildl. Dis.*, 2014, vol. 50, no. 2, p. 297. https://doi.org/10.7589/2012-02-047
77. Curry, P.S., Elkin, B.T., Campbell, M., Nielsen, K., Hutchins, W., Ribble, C., and Kutz, S.J., *J. Wildl. Dis.*, 2011, vol. 47, no. 1, p. 12. https://doi.org/10.7589/0090-3558-47.1.12
78. Jara, R.F., Sepulveda, C., Ip, H.S., and Samuel, M.D., *J. Wildl. Dis.*, 2015, vol. 51, no. 2, p. 475. https://doi.org/10.7589/2013-07-185
79. Nemeth, N.M., Bosco-Lauth, A.M., Williams, L.M., Bowen, R.A., and Brown, J.D., *Vet. Pathol.*, 2017, vol. 54, no. 6, p. 901. https://doi.org/10.1177/0300985817717770
80. Saushkin, N.Yu., *Cand. Sci. (Chem.) Dissertation*, Moscow: Moscow State Univ., 2019.
81. Osipov, A.P., Kondakov, S.E., Grigorenko, V.G., Smolenskii, V.I., Prokoptseva, O.S., and Samsonova, Zh.V., RF Patent 2519030, 2014.
82. Samsonova, J.V., Chadina, A.D., Osipov, A.P., and Kondakov, S.E., *Anal. Methods*, 2016, vol. 8, p. 4835. https://doi.org/10.1039/C6AY00724D
83. Samsonova, J.V., Osipov, A.P., and Kondakov, S.E., *Talanta*, 2017, vol. 175, p. 143. https://doi.org/10.1016/j.talanta.2017.07.032
84. Samsonova, J.V., Osipov, A.P., and Kondakov, S.E., *Vet. J.*, 2014, vol. 199, no. 3, p. 471. https://doi.org/10.1016/j.tvjl.2013.10.031
85. Samsonova, J.V., Saushkin, N.Yu., Osipov, A.P., Kondakov, S.E., Fomina, S.N., and Mischenko, A.V., *Appl. Biochem. Biotechnol.*, 2019, vol. 188, p. 491. https://doi.org/10.1007/s12010-018-02938-3
86. Saushkin, N.Yu., Samsonova, J.V., Osipov, A.P., and Kondakov, S.E., *J. Virol. Methods*, 2019, vol. 263, p. 101. https://doi.org/10.1016/j.jviromet.2018.11.004
87. Saushkin, N.Yu., Samsonova, Zh.V., Osipov, A.P., Kondakov, S.E., Lysova, E.S., Elizarova, I.A., Khaertynov, K.S., Shuralev, and E.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2018, vol. 73, no. 3, p. 135. https://doi.org/10.3103/S0027131418030069
88. Chvala, I.A., Mishchenko, A.V., Lozovoi, D.A., Saushkin, N.Yu., Samsonova, Zh.V., Kondakov, S.E., Osipov, A.P., Kuznetsov, D.V., and Chernykh, O.Yu., *Veterinariya*, 2018, no. 2, p. 24.
89. Saushkin, N.Yu., Samsonova, Zh.V., Osipov, A.P., Kondakov, S.E., Efimova, M.A., and Chernov, A.N., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2016, vol. 71, no. 4, p. 253. https://doi.org/10.3103/S0027131416040088
90. Saushkin, N.Yu., Samsonova, Zh.V., Osipov, A.P., Kondakov, S.E., Khammadov, N.I., Usol'tsev, K.V., Makaev, Kh.Z., and Chernov, A.N., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2016, vol. 71, nos. 5–6, p. 319. https://doi.org/10.3103/S0027131416050084
91. Samsonova, Zh.V., Saushkin, N.Yu., Osipov, A.P., Yakovlev, S.S., and Rozhdestvenskaya, T.N., *Ptitsa Ptitseprod.*, 2019, no. 5, p. 48.
92. Saushkin, N.Yu., Samsonova, Zh.V., Osipov, A.P., and Serebrennikova, K.V., RF Patent 2706405, 2019.
93. Trifonova, O.P., Maslov, D.L., Balashova, E.E., and Lokhov, P.G., *Metabolites*, 2019, vol. 9, no. 11, p. 277. https://doi.org/10.3390/metabo9110277
94. Bhuiyan, A.R., Chowdhury, E.H., Kwiatek, O., Parvin, R., Rahman, M.M., Islam, M.R., Albina, E., and Libeau, G., *BMC Vet. Res.*, 2014, vol. 10, p. 247. https://doi.org/10.1186/s12917-014-0247-y
95. McDade, T.W., Williams, S.A., and Snodgrass, J.J., *Demography*, 2007, vol. 44, no. 4, p. 899. https://doi.org/10.1353/dem.2007.0038
96. Lange, B., Roberts, T., Cohn, J., Greenman, J., Camp, J., Ishizaki, A., Messac, L., Tuaillon, E., van de Perre, P., Pichler, C., Denkinger, C.M., and Easterbrook, P., *BMC Infect. Dis.*, 2017, vol. 17, no. 1 (suppl.), p. 693. https://doi.org/10.1186/s12879-017-2776-z
97. Rowland, M. and Emmons, G.T., *AAPS J.*, 2010, vol. 12, no. 3, p. 290. https://doi.org/10.1208/s12248-010-9188-y
98. Henion, J., Oliveira, R.V., and Chace, D.H., *Bioanalysis*, 2013, vol. 5, no. 20, p. 2547. https://doi.org/10.4155/bio.13.197
99. la Marca, G., Malvagia, S., Pasquini, E., Innocenti, M., Fernandez, M.R., Donati, M.A., and Zammarchi, E., *Rapid Commun. Mass Spectrom.*, 2008, vol. 22, p. 812. https://doi.org/10.1002/rcm.3428
100. Blau, N., *Hum. Mutat.*, 2016, vol. 37, no. 6, p. 508. https://doi.org/10.1002/humu.22980
101. Karstad, L., Spalatin, J., and Hanson, R.P., *J. Infect. Dis.*, 1957, vol. 101, no. 3, p. 295.
102. Lehner, A.F., Rumbeiha, W., Shlosberg, A., Stuart, K., Johnson, M., Domenech, R., and Langner, H., *J. Anal. Toxicol.*, 2013, vol. 37, no. 7, p. 406. https://doi.org/10.1093/jat/bkt048
103. Shlosberg, A., Rumbeiha, W.K., Lublin, A., and Kannan, K., *J. Environ. Monit.*, 2011, vol. 13, no. 6, p. 1547. https://doi.org/10.1039/C0EM00754D
104. Shlosberg, A., Wu, Q., Rumbeiha, W.K., Lehner, A., Cuneah, O., King, R., Hatzofe, O., Kannan, K., and Johnson, M., *Arch. Environ. Contam. Toxicol.*, 2012, vol. 62, no. 3, p. 502. https://doi.org/10.1007/s00244-011-9709-4
105. Lehner, A.F., Johnson, M., and Buchweitz, J., *Toxicol. Mech. Methods*, 2017, vol. 28, no. 1, p. 29. https://doi.org/10.1080/15376516.2017.1354414
106. Perkins, M. and Basu, N., *Environ. Pollut.*, 2018, vol. 236, p. 236. https://doi.org/10.1016/j.envpol.2018.01.036
107. Posyniak, A., Zmudzki, J., and Niedzielska, J., *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2002,

vol. 780, no. 2, p. 309.
https://doi.org/10.1016/S1570-0232(02)00540-8

108. AhMed., H.A., Picozzi, K., Welburn, S.C., and MacLeod, E.T., *Parasites Vectors*, 2013, vol. 6, no. 1, p. 316.
https://doi.org/10.1186/1756-3305-6-316

109. Adams, E.R., Hamilton, P.B., Malele, I.I., and Gibson, W.C., *Infect., Genet. Evol.*, 2008, vol. 8, no. 4, p. 439.
https://doi.org/10.1016/j.meegid.2007.07.013

110. Hall-Mendelin, S., Ritchie, S.A., Johansen, C.A., Zborowski, P., Cortis, G., Dandridge, S., Hall, R.A., and Hurk, A.F., *Proc. Natl. Acad. Sci. U. S. A.*, 2010, vol. 107, p. 11255.
https://doi.org/10.1073/pnas.1002040107

111. Brito, C.M., Lima, M.M., Sarquis, O., Pires, M.Q., Coutinho, C.F., Duarte, R., and Pacheco, R.S., *Parasitol. Res.*, 2008, vol. 103, no. 5, p. 1111.
https://doi.org/10.1007/s00436-008-1102-5

112. Hopkins, J.S., Chitambo, H., Machila, N., Luckins, A.G., Rae, P.F., van den Bossche, P., and Eisler, M.C., *Prev. Vet. Med.*, 1998, vol. 37, nos. 1–4, p. 91.
https://doi.org/10.1016/S0167-5877(98)00101-9

113. Rasolonjatovo, F.S., Guis, H., Rajeev, M., Dacheux, L., Arivony Nomenjanahary, L., Razafitrimo, G., Rafisandrantantsoa, J.T., Cêtre-Sossah, C., Heraud, J.M., and Andriamandimby, S.F., *PLoS Neglected Trop. Dis.*, 2020, vol. 14, no. 3, e0008116.
https://doi.org/10.1371/journal.pntd.0008116

114. Foss, L., Reisen, W., Fang, Y., Kramer, V., and Padgett, K., *PLoS One*, 2016, vol. 11, e0157555.
https://doi.org/10.1371/journal.pone.0157555

115. Al-Kappany, Y.M., Abbas, I.E., Devleesschauwer, B., Dorny, P., Jennes, M., and Cox, E., *BMC Vet. Res.*, 2018, vol. 14, no. 1, p. 120.
https://doi.org/10.1186/s12917-018-1440-1

116. Jefferies, R., Ryan, U.M., and Irwin, P.J., *Vet. Parasitol.*, 2007, vol. 144, nos. 1–2, p. 20.
https://doi.org/10.1016/j.vetpar.2006.09.022

117. Pedersen, L., Andersen-Ranberg, K., Hollergaard, M., and Nybo, M., *Clin. Biochem.*, 2017, vol. 50, no. 12, p. 703.
https://doi.org/10.1016/j.clinbiochem.2017.01.010

118. Liu, G., Mühlhäusler, B.S., and Gibson, R.A., *Prostaglandins, Leukotrienes Essent. Fatty Acids*, 2014, vol. 91, no. 6, p. 251.
https://doi.org/10.1016/j.plefa.2014.09.009

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