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CARDS FOR COLLECTION OF BIOMATERIAL SAMPLES: AREAS OF APPLICATION

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ABSTRACT

Cost-efficient methods for the collection and storage of biological samples are currently in great demand in the life sciences field. The storage of material on a filter paper carrier has been recently introduced as an option. The carrier is a chemically-treated sample collection matrix that lyses cells and preserves DNA spotted onto the paper. A similar paper-based collection of DNA samples is currently used in many areas of research due to its simplicity, safety, and affordable price. Nowadays, a variety of biological samples are suitable for collection with this carrier, including not only blood and buccal epithelial cells but also bacterial, plant, and insect biomaterial. This review will cover the latest application areas of the paper-based biomaterial collection in areas such as healthcare, microbiology, and agriculture.

Keywords: DNA, identification, buccal cells, filter paper, PCR

INTRODUCTION

At present, polymorphic DNA markers, which have been identified by deciphering the human genome, are powerful tools for carrying out diverse genetic analysis. For example, one of the main approaches to the study of the genetic structure of modern populations and its genetic history is the analysis of Y-chromosome haplogroups based on the genotyping of DNA markers, as well as the analysis of the mtDNA haplogroups on the basis of determining the non-coding primary nucleotide sequence [1-3].

In addition to the study of the genetic structures of various populations, tremendous progress was made in the conduct of biometric and genetic certification of residents of different countries. In the US, the CODIS DNA database, which means the Combined DNA Index System, was launched in 1994 [4, 5]. In Russia, genomic registration has been in effect since 2009. Russian specialists have been convinced of the high efficiency of genomic registration as a means of fighting crime, international terrorism, drug business, as well as many other modern threats to society and the state [6].

In December 2016, the President of the Republic of Kazakhstan signed the law "On fingerprinting and human genetic identity testing". The purpose of this law is to establish or confirm identity on the basis of fingerprint and genomic registration (profiling). According to the law, those convicted of certain types of crimes are subjects to genomic registration, as well as unidentified persons whose biological material is

seized during investigative actions. Genomic information will be used to prevent, solve and investigate a crime, as well as identify the perpetrators.

From the technical point of view, the implementation of this law will result in significant increase in the number of DNA analyses and examinations conducted by the departments of the Ministry of Internal Affairs and the Ministry of Justice. The law will come into force in 2021 in Kazakhstan. This will entail not only significant expansion of the existing technical facilities but also a large-scale acquisition of foreign consumables, both for the collection of biomaterial and for genetic profiling [7, 8].

In this regard, one of the promising areas of biotechnology is the production of laboratory consumables for genetic analysis, in particular, kits for collection and storage of biological material, compatible with genetic human identity testing [9]. FTA™ Cards (GE Healthcare, USA) and Nucleic-Cards™ (Copan, Italy) are the leaders in this field providing simultaneous cell lysis and protein denaturation [10, 11].

It should be noted that with the development of PCR and sequencing, forensic scientists have established a new branch of science called DNA forensics. This paper provides an overview of published works in the field of genetics, forensics, and biomedicine ending with the discussion on the future use and storage of biomaterial.

Areas of application

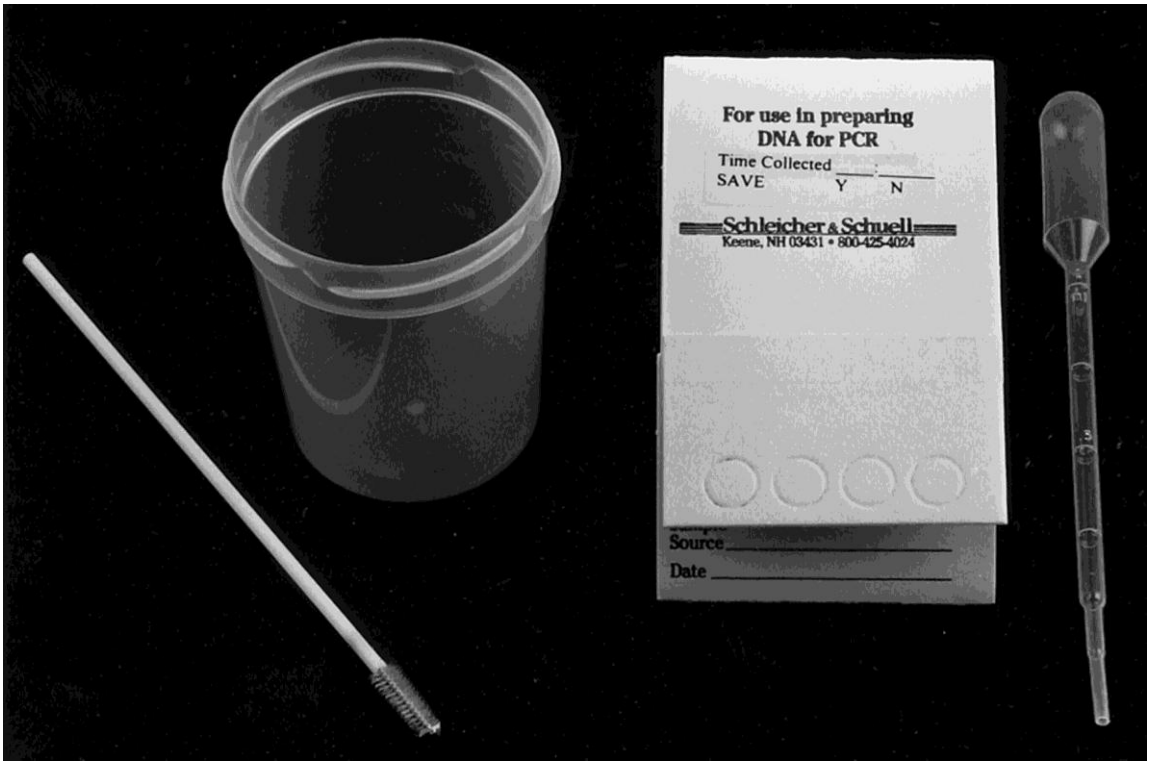
The need for DNA amplification puts in the first place the stage of extraction and storage of genetic material. With the development of DNA-based analysis, there is a need for effective, fast and economical methods for the isolation and storage of the biomaterial. Traditional storage methods are freezing of DNA in solution and further storage at temperatures from -20 °C and below to prevent degradation of nucleic acids.

A new method of dry DNA storage has emerged in 1963. The idea started with a method paper by Guthrie R and Susi A who applied blood obtained by heel puncture to a piece of absorbent filter paper to detect phenylketonuria in newborns [12]. The filter paper has been further developed and improved to lyse cells and stabilize NA by impregnated chemicals [13]. For long-term dry-state storage of the biomaterial at room temperature, universal paper carriers based on different porous materials have been developed. There are various commercial kits for DNA extraction based on this technology, such as Whatman™ FTA™ (Flinders Technology Associates) Card (GE Healthcare, USA), Nucleic-Card™ (Copan, Italy), Buccal DNA Collector (Bode Technology, USA) and DNA-Card (Alcor Bio, Russia). These cards are impregnated with a patented mixture of chemicals that make up the lysing solution to destroy cells, prevent the growth of bacteria and protect DNA from nucleases [14]. These cards are widely applicable in forensic science, diagnostics, as well as in the collection of biomaterial in the field [15].

DNA cards come in different configurations. The full package usually contains several levels of protection such as barcoding, protective stickers, and sample collection kit. Sample collection kit may include disposable gloves, sterile wipes, and a blood drawing device. There are also options without a blood drawing device. These cards are designed for research work in a variety of areas related to DNA analysis. There are specific DNA cards intended for the collection of buccal epithelium, which is contained in saliva. This card is additionally impregnated with a special dye that becomes discolored when applying the biomaterial, which allows visualizing the place of application of the sample. In addition, the DNA cards are equipped with a convenient plastic holder with an applicator.

There are several types of FTA cards that differ in the scope of the sample use, for example, for collecting blood, buccal epithelial cells, special cards for analyzing microorganisms, plants and insects [14, 16]. All of them are characterized by the speed of collection, efficiency, and safety of DNA, which is necessary for high-quality genetic

analysis. One of the most accessible and non-invasive materials for human genotyping is buccal cells (fig. 1). It was shown that when buccal cells are collected on the FTA kit, the yield of genomic DNA from buccal cells was about 10 ng [17]. The collection of epithelial cells is carried out using a special brush, which is then applied to the card to transfer cells. As a rule, any negatively charged molecules in high concentration can push nucleic acids out of solution and enhance their binding to cellulose paper. In addition, FTA cards contain chemicals that promote cell lysis, DNA binding to the surface, and protection against degradation [18].



Subjects brushed their oral mucosa with the soft-bristled cytobrush, swished the saliva that collected in their mouths during brushing, and expectorated it into the cup. The saliva sample was transferred to the card (filter paper) using the disposable pipette.

Fig. 1. Paper-based buccal cell collection kit [10].

In the case of FTA cards, the manufacturer guarantees the safety of dry blood on an FTA-carrier for at least 20 years (in terms of DNA preservation) [3, 4]. In addition, the use of FTA-paper as a carrier material for blood samples allows not only long-term storage of these samples but also easily and quickly purify DNA from impurities of proteins and iron-containing compounds. DNA bound to the FTA matrix is released from heme and other inhibitors in the polymerase chain reaction (PCR) by simple washing with a proprietary FTA reagent. After that, FTA-paper with DNA immobilized on it can be added directly into the amplification mixture.

A number of researchers reported a lack of effectiveness of FTA-cards during long-term storage of biological samples [19]. In particular, during 7-year storage of whole blood on FTA-cards at 37 °C, most likely, degradation of high-molecular STR-loci occurs. DNA of the buccal epithelium after 7 years of storage on FTA-carrier at room temperature also does not retain its original qualitative and quantitative characteristics [11]. On the other hand, the work of a number of authors reports the positive experience of using FTA cards in the DNA analysis [20, 21].

It should be noted that FTA cards are a more reliable method of collecting DNA for PCR than brushes for buccal epithelium. Successful amplification was observed

about 20% more often in samples isolated with cards. An additional advantage of the cards is long-term storage compared to brushes for collecting buccal epithelium, for which the recommended storage is no more than 5 days [22]. The collected material can be stored at room temperature as long as 20 years as stated by the manufacturers. Studies of dried blood samples were performed, followed by a successful PCR analysis after 4 and 8 years of storage. To extract the nucleic acid from the card, cut out two round disks (3-6 mm), the surface area of the card allows you to get approximately 25 disks. The impregnating chemical solution contains a number of components of PCR inhibitors, which are removed by washing steps with a cleaning reagent and TE buffer. In this step, DNA is tightly bound to the surface of the disk, while proteins and inhibitors are washed away. After washing the discs must be dried at room temperature, add reagents to the PCR in the same tube.

Another study has confirmed a successful analysis of samples of dry blood immobilized on the FTA cards following a 15-year period of their storage at room temperature [23]. At the first stage of work, the efficiency of the sample preparation method recommended by the manufacturer of FTA cards, namely washing the immobilized DNA with the help of an FTA reagent, was evaluated. The STR-typing of the control 2-day blood samples revealed complete genetic profiles with AmpFlSTR Identifiler Plus PCR Amplification Kit (Life technologies) panel for the entire control group of 22 people. Of the 50 FTA cards that were stored at room temperature (24-28 °C) for 15 years, it was possible to obtain the full genetic profile for 41 (82%) out of 50. Noteworthy, in all 50 samples, the amplification efficacy in high-molecular STR-loci (over 300 bp long) was significantly lower than low-molecular STR-loci (less than 300 bp). In 9 (18%) out of 50 samples, after standard washing with an FTA reagent of 15-year-old FTA cards, incomplete genetic profiles were obtained, in which the absence of PCR products, the loss of true and the inclusion of false alleles were observed for some loci. No correlation with the sizes of the amplified STR-loci was observed. The absence of such a correlation suggests that the cause of PCR artifacts is not the degradation of the DNA template, but the residual presence of inhibitors of DNA polymerase activity as a result of incomplete purification. Indeed, even a visual assessment indicated that 15-year-old FTA cards were washed off with an FTA reagent markedly worse than 2-day samples.

The reason for the unsatisfactory results may be the residual presence of inhibitors of DNA polymerase activity, due to the inefficient washing. In the case of long-stored samples, it is preferable to use instead of the recommended FTA washing solution, alternative techniques taking care of total extraction of DNA immobilized on FTA-cards, with its subsequent effective washing. The obtained results demonstrate that, in particular, the quantity and quality of DNA in preparations obtained from blood samples stored on FTA-cards for 15 years is quite acceptable for routine forensic typing of STR-loci of chromosomal DNA.

Biomedicine

The simplicity, efficiency and short duration of the method, the economics of using the minimum amount of reagents promoted the use of cards in numerous studies. For example, FTA cards were successfully used for the genotyping of tumor cells [24, 25]. Blood from each subject, which was then transferred onto collection cards, were stored at room temperature, and there was no correlation between the storage time and the deterioration of the amplification. Stability of tumor cell DNA in cells stored on cards was inspected from 10 to 150 days [26]. No PCR failures were seen in specimens stored for more than 80 days. Comparison of the results of the amplicon intensity between groups of specimens stored on FTA cards for increasing lengths of time

showed no obvious trends, with intermediate to strong bands seen in most of the samples tested.

In another study FTA technology was successfully used for collection, archiving, and molecular analysis of microsporidia DNA from clinical stool samples [27]. The FTA technology was applied for sampling, archiving, and molecular analysis of the DNA isolated from stool samples to diagnose and identify microsporidia, the intracellular opportunistic parasites which induce malabsorption syndrome in immunosuppressed humans, particularly in patients with AIDS. Microsporidia DNA was successfully amplified in 6 of 50 stool samples of HIV-positive patients of the S. P. Botkin Memorial Infectious Disease Hospital (St. Petersburg) applied to FTA cards. The FTA method of DNA immobilization is especially promising for epidemiological and field population studies which involve genotyping of microsporidia species and isolates.

Successful use of cards for pharmacogenetic studies has been shown on several types of genotyping: multiplex PCR, PCR restriction fragment length polymorphism and analysis of allelic discrimination of genes. DNA for the study was isolated from blood, the results showed the presence of amplification in the samples, regardless of the method of genotyping [15].

Agriculture

The quality of bovine genomic DNA after isolation on FTA cards was evaluated using the Illumina Bovine SNP50 iSelect BeadChip, where for a successful experiment, the fragment size should be ≥ 2000 bp. Blood samples and nasal cattle excretions were collected on cards, in parallel, for comparison, DNA extraction was carried out using the phenol-chloroform method. There was no significant difference between the genotypes of the samples, the authors of the study concluded that the FTA kit provides acceptable DNA quality for the application of Illumina iSelect technology [28].

The effectiveness of the kit was also evaluated in the poultry industry on cultures of *Campylobacter jejuni*. In order to determine drug resistance, the cell suspension was applied to FTA cards and stored at room temperature for three months until DNA extraction. Amplification by the *gyrA* gene, which is a genetic marker of resistance, was identified in all the samples studied. According to the results, DNA retained its integrity after three months of storage at room temperature, which indicates the successful use of cards for the genotyping of bacterial cultures [29].

FTA cards could be used to transport DNA samples from other pathogenic bacteria, reducing biohazards associated with shipping live cultures. The possibility of shipping DNA, in an economic and safe way, for testing samples at the laboratories facilitated the identification of *Salmonella enterica* serotypes that were circulating in the environment of poultry was also reported [30]. The FTA cards provided safe and effective inactivation of the pathogen, and the DNA obtained from the cards were adequate for downstream analyses.

Extraction of nucleic acids

Extraction of the nucleic acids from the sample includes the following steps: (a) lysis of the sample containing the nucleic acid to obtain a lysate solution or (b) binding of the nucleic acid to the matrix; and (c) washing and eluting a matrix-related nucleic acid to isolate and purify nucleic acids. Rapid purification of nucleic acids from inhibitors can take place in several stages, while the DNA remains on the matrix and is ready for amplification.

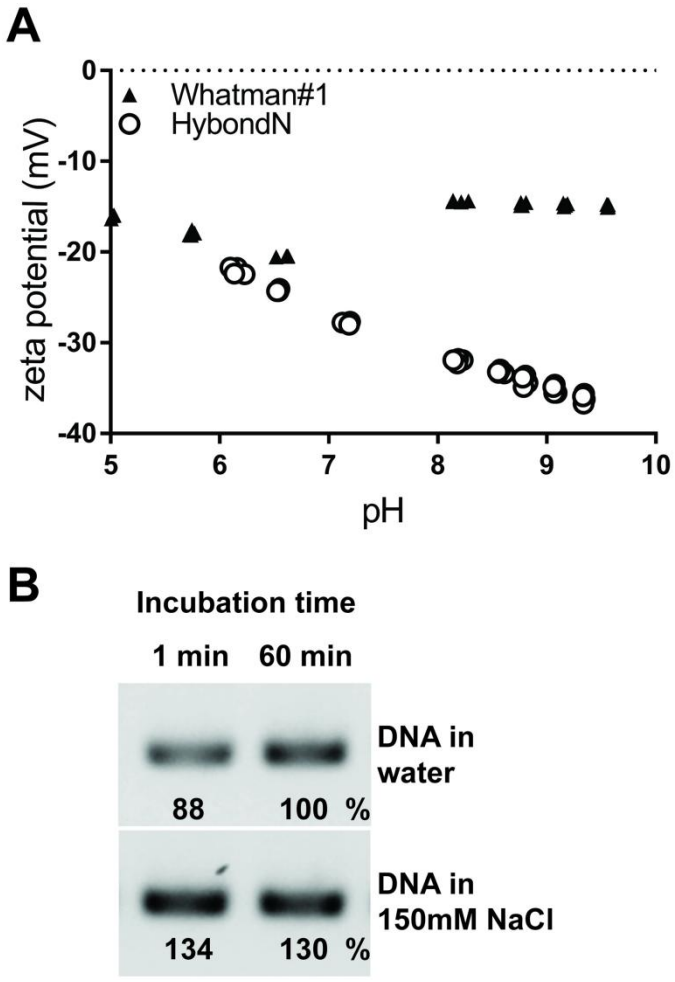
Lysis of cells is usually achieved through impregnation of porous carriers used for the collection and storage of biological material, ensuring the stability of the DNA of biological samples in a wide range of temperatures for long periods of time.

Standard CTAB buffer (2% CTAB, 100 mM Tris-HCl, pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl) was used to lyse the plant cells that were disrupted using a TissueLyser (Qiagen) [31]. CTAB buffer also contained freshly added RNase A (20 ng/ml final concentration). After CTAB buffer treatment filter paper absorbs DNA from sample lysate solution since a high concentration of NaCl in lysis buffer provides a chaotropic condition for DNA binding. DNA binding efficiency depends on the physical properties of the binding material (table 1).

Table 1. DNA yield using different DNA binding materials [31]

DNA binding materials	DNA yield (ug)
Whatman™ qualitative filter paper, Grade 3	7.5 ± 0.6
Fisherbrand™ qualitative grade plain filter paper, Grade P4	5.8 ± 0.3
Fisherbrand™ qualitative grade plain filter paper, Grade P8	2.9 ± 0.2
Whatman™ glass microfiber filters, Grade GF/F	3.1 ± 0.7
Fisherbrand™ glass fiber, Grade 8	2.8 ± 1.2
Fisherbrand™ glass fiber, Grade 2	4.0 ± 0.7

It was found that the surface of the filter paper has a negative zeta potential that remains relatively constant across the range of pH (pH 5 to pH 10). As DNA also carries a net negative charge, largely due to its electronegative phosphate backbone, the like charges between the DNA and the filter paper surface will result in a repulsive force that will hinder DNA binding. Increase in DNA amplification was observed from samples diluted in 150 mM NaCl compared to those diluted in water by counteracting the electrostatic repulsion (fig. 2) [18]. Other compositions of the plant cell lysis buffer contained SDS, guanidine hydrochloride or Tween 20 instead of CTAB have been reported [18]. In addition, SDS can be replaced by N-lauryl sarcosine which is the soft detergent that does not lead to inhibition of PCR on the next step.



A - The zeta potential of filter paper (black triangles) and nylon membrane (open circles) was measured across a range of pHs. B - Paper was incubated in plant genomic DNA (1 ng/µl) dissolved in water or in 150 mM NaCl. The DNA solution was removed from paper discs by spinning, and the discs were added to PCR amplification. The band intensities achieved with cellulose discs relative to the 60-minute sample in water appear below each band.

Fig. 2. Salts enhance DNA binding [18]

The common constituent of the lysis buffer is 5-20 mM EDTA. Due to the presence of EDTA in the buffer, the inhibitory effect of Fe^{2+} and Fe^{3+} ions on PCR is eliminated. It is known that weak alkaline pH is optimal for storing DNA. Therefore, a 30–200 mM Tris solution (Tris (hydroxymethyl) aminomethane) is used to create a buffer capacity and maintain a slightly alkaline pH. Sodium azide can also be a part of the buffer compositions since it has a pronounced antimicrobial and antimycotic effect without inhibiting PCR.

After successful binding, proteins and contaminants can be washed off using 70% ethanol then DNA can be eluted using TE buffer (10 mM Tris-HCl, 2 mM EDTA, pH 9.0) incubating ten-fifteen minutes at 94 ^{o}C. Alternative wash buffer is 10 mM Tris (pH 8.0), 0.1% Tween-20 [18]. TE buffer and water are the most common elution buffers to elute DNA from the filter paper. The elution step may be omitted by directly amplifying the nucleic acid of the membrane [18]. This gives an advantage over of the solid-phase extraction techniques as the residual reagents (e.g., ethanol, chaotropic salts) may inhibit DNA amplification.

As a result, filter paper-based method for nucleic acid extraction has several advantages. First, filter paper rapidly absorbs a sufficient amount of nucleic acids through capillary action. A sufficient amount of nucleic acid is retained on the matrix even after extended incubation in a large volume of water, while inhibitors including Proteinase K and other proteins can be easily eluted. Filter paper enables elution of a

sufficient quantity of bound nucleic acids into the amplification mix. This rapid elution is likely catalyzed by dNTPs present in the amplification mix as has been reported in several studies [18].

CONCLUSION

Paper-based collection of DNA samples has been introduced to many areas of research due to its simplicity, effectiveness, safety and affordable price. The entrapped nucleic acids are protected from degradation for a long period of time at room temperature. Nowadays, paper cards are mostly applied to the collection of blood and buccal epithelial cells. However, a range of biological samples, suitable for the collection, has been extended to microorganisms, plants, and insects.

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