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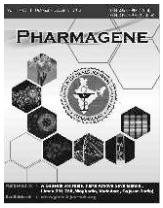

"Dry Blood Spot Technique: A Review"

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Abstract:

Over the past several years dried blood spot (DBS) sampling technique has been emergedas a relevant method in both qualitative and quantitative bioanalysis. In DBSmethod, blood sample is directly spotted on to a paper (with or without treatment) and afterdrying it can be analyzed by modern analytical, immunological or genomic detectionsystems. Several advantages of DBS technique such as low blood volume requirement,transportation and storage without special treatment, better analytes stability, enhancedclinical cooperation in clinical trials and reduced exposure of biohazard to analysts, make it the most appropriate blood samplingtechnique.DBS method explores the proficiency and appliance of DBS method in Pharmacokinetic (PK), Therapeutic drug monitoring (TDM), Toxicokinetic (TK), Metabolomic, Disease diagnosis and Epidemiological studies.The current article is focused on the available information on DBS method which may serve as a single framework for investigatorsin the field of bioanalysis.

Key Words : Dried Blood Spot (DBS), Pharmacokinetics (PK), Therapeutic Drug Monitoring (TDM), Preclinical study

Introduction :

Dried blood spots (DBS) refers to a blood sampling technique where small volumes of bloodare spotted on an appropriate filter paper, dried, and then taken to the labratory for analysis.Thetechnique is well established in clinical labs for applications such as neonatal screeningfor inborn diseases, but has recently experienced a increase of interest in the area of drugdevelopment, i.e. toxicokinetic and pharmacokinetic studies.

Dried Blood Spots (DBS) are whole blood was collected on filter paper and dried. DBS sampling approaches for drug development are amenable to the same types ofbioanalytical detection principles as traditional plasma or whole blood samples, i.e. HPLC-UV, LC/MSMS, GC/MS. Microbiological assays used based on DBS sampled material for folate screening.Thyroid hormones have been analyzedby immunoanalytical methods in neonatal screening, which indicates that the technique is capable of being to ligand binding approaches.Various biomarkers and even immunogenicity assays have been performed with the DBS,but most DBS work was based on filterpapers that contained denaturing agents, inactivating bacteria and viruses, which contributesto safety and stability. However,thesetypes of filter paper denature proteins, and as a consequencemay destroy epitopes necessary for binding in a ligand binding assay, or render the inaccessible. It is therefore recommended to use non-denaturing filter paper and solvents that are compatible with protein work when attempting to quantitate proteinaceous compounds (biopharmaceuticals, biomarkers, antibodies) in DBS[1].

DBS micro volume sampling using specialized Whatman media from GE Healthcare has been shown to be precise and accurate for a variety of compounds from different structural classes with acceptable inter- and intra-assay variability.

DBS are being used for re-testing at a reference laboratory, which may be part of country's External Quality Assessment plan. Testing site results are being compared to reference laboratory results. DBS samples are useful for re-testing as they are easy to collect, store and transport.

DRY BLOOD SPOT TECHNIQUE PROCEDURE
COLLECTION AND SAMPLING

The collection area (finger, heel) has to be first disinfected. The skin is then punctured with a sterile lancet (Figure 1). The blood drops are placed on blotting paper marked with circles to be filled. Once all the required circles are filled, the blotting paper is left to dry for a few hours at room temperature on a non-absorbent surface.The sample is punched (2–6 mm) and the analytes are extracted using an appropriate buffer before analysis.

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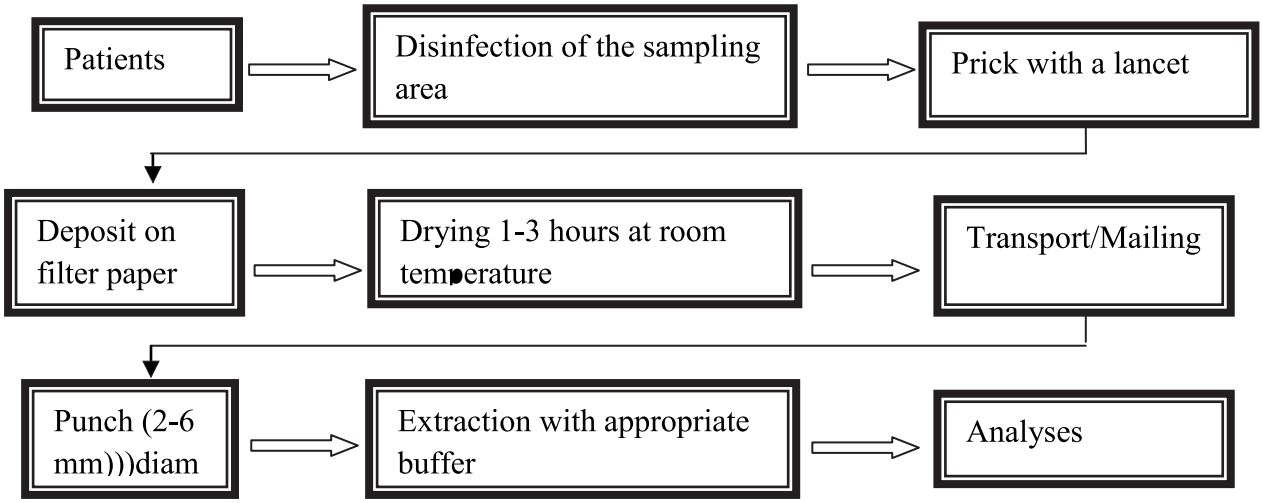

Figure 1: DBS collection process.

CONSERVATION

Once dry, the DBS cards are shifted into a waterproof plastic bag, possibly along with a desiccant and a humidity indicator. The purpose of the desiccant is to finalize the drying process, which also minimizes any risk of infection associated with sampling. Periods of storage at room temperature vary according to the biological factor, from 1 week for proteins, to 1 year or more for nucleic acids. As far as serology is concerned, the blotting papers are usually being kept at –20°C upon receipt. For long-term preservation (up to several years) the blotting papers are stored either at −20°C or –80°C[2].

EXTRACTION

Extraction of the analytes from DBS specimens are needs to be achieved using a standard procedure. One or more 2–8 mm diameter discs were then created with a specific punch. These small "spots" was placed in an elution buffer for variable time space according to the procedure. The DBS extraction is then treated as a hemolyzed whole blood sample, and tested with methods often intended for plasma or serum. The elution buffer plays a major role in re-solubilizing the analytes to be tested. The most common are saline/phosphate buffers, often with added detergents (Tween, Triton,etc), carrier proteins and chelators (ethylene diamine tetra acetic acid EDTA), as well as organic buffers with methanol, acetonitrile or ethanol.

PROS AND CONS OF DBS

One of the important advantages of using DBS technology is that it allows access to samples in pre analytical situations were standard blood collection is challenging (problem with sampling, storage). The typical DBS contains approximately 50 µL of whole blood on an average surface of 12 mm2 (Figure 2). It makes possible the testing of various analytes such as nucleic acids, proteins, lipids, or small organic and non-organic molecules. Two types of DBS are mostly available: cotton paper filters of different qualities (Whatmann 903 Protein Saver Cards Whatmann, Springfield Mill, UK; Perkin Elmer 226 Spot Saver Card, Perkin Elmer, Waltham, USA) and glass microfiber filter papers (Agilent Bond Elut DMS, Santa Clara, CA, USA; Sartorius Glass Microfiber Filters, Goettingen, Germany). The main difference between the two supports is that the glass fiber does not soak up reagents, which diminishes non-specific analyte adsorption on the membrane[3].

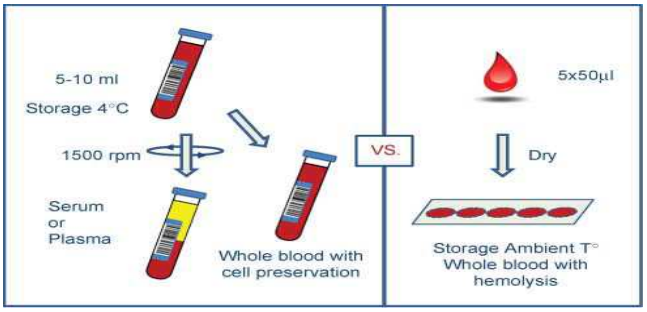

Figure 2: Comparison of the use of classical blood sampling vs DBS sampling resulting in a 100-fold reduction in blood volume.

DBS collection is easy to perform and relatively painless (Figure 1). It can be carried out by the patient at home, without the need of medical laboratories. This sampling procedure is far less invasive than veinpuncture; whichis better suited for patients requiring several blood tests, such as those with damaged/altered veins, the elderly or infants. The use of DBS also minimizes the volume of blood is taken from patients. It has been shown that drying the blood spot on blotting paper damages the capsid of viruses [HIV, Cytomegalovirus(CMV), hepatitis C virus(HCV), human T-lymphotropic virus (HTLV)] reducing any possible risk of contamination for medical or paramedical staff. In addition, it enables the shipping of samples by regular mail with no particular risk of contamination. This indicates a valuable asset for sampling in remote communities either located far away from a testing laboratory or with limited technical infrastructure available, therefore provides added value compared to standard blood sampling. Through its small size and stacking capacity, DBS is also used for reducing and facilitating storage in clinical laboratories and biobanks4.DBS have been utilized in experimental research, by facilitating pharmacological studies and pharmacokinetics on small animals with very limited volumes of biological liquids. This follows the regulations aimed at protecting small animals (decreasing sample volume and sophistication of sampling methods) during pre-clinical studies. Concerning sample stability, many studies have been shown that most analytes from whole blood are stable at room temperature for at least 7 days. In some cases such as opiates, DBS even surge stability during storage,

and nucleic acids are a major tool for short- and long-term preservation, as they can be isolated after several months at room temperature and several years at −20°C. From a medico-economical point of view, the use of DBS allows a significant cost reduction due to decreased requirements in trained staff, facilitated transportation, storage, and processing.

A major drawback of DBS technology resides in the nature of the biological sample itself (Figure 2). In a standard sampling procedure, either serum or plasma is being analyzed, whereas DBS samples are composed of hemolyzed whole blood5. Hence, obstructing due to hemoglobin and the release of intracellular content could occur. The blood cells (erythrocytes, leukocytes, platelets etc.) are altered by the drying process, thus cellular hematological testing is impossible. Drying can also denature proteins and alters the enzymatic activity of blood proteins (aspartate transaminase).The small volume of samples resulting from the DBS can be a disadvantage for low sensitivity assays and for running multiple tests.

APPLICATIONS

Typical fields of applications are:

Newborn Screening

Many recent applications inthe newborn screening using LC-MS/MS have been demonstrated that the technique is a better choice thanMS alone or some other means for the accuratequantification of biomarker molecules in the DBS samples6. There are many situations in newborn screening whereabsolute concentration of a single biomarker moleculemay not be diagnostic; instead, accurate quantification ofmultiple biomarker molecules using LC-MS/MS, followedby comparison of response ratios of one species to the other(s), is more powerful. Eg.,The screening for congenitaladrenal hyperplasia (CAH). 17-Hydroxyprogesterone (17-OHP) is one of the biomarkermolecules for the early diagnosis of CAH. Thedevelopment of an immunoassay; to determine the 17-OHP concentration in blood spots in newborn screening, was introduced in 1977. Due to aphysiologically delayed expression of the enzyme 11β-hydroxylase in premature babies; illness, birth stress,impaired kidney function, interference from 17-OHPmetabolite(s) and cross-reactivity of antibodies with othersteroids particularly 17-hydroxypregnenolone (17-OHPreg), 17-OHP has amolecular weight of 330 Dalton (Da),2 Da less than thatof the interfering compound 17-OHPreg (332 Da). Bothcompounds can be distinguished by mass spectrometryvia any detection mode (Q1scan, product scan, or selected reaction monitoring).

Preclinical Study

DBS in preclinical study have been published on toxicokinetic or pharmacokinetic assessment of drug or drug candidates in small animals. In all cases where serum or plasma, instead of DBS, has been used as the matrix for quantitative analysis of drugs during discovery and development, it would be necessary to determine the relationship between the analyte concentration in the two matrices (plasma/serum V/s DBS). Without correcting for its impact, the analytical results from DBS would be much lower than those obtained via plasma or serum, regardless of which assay might have been used for the analysis of plasma or serum samples[7].

Therapeutic Drug Monitoring

Therapeutic drug monitoring (TDM) is a major field for the DBS technology, since drawing blood samples can be performed by the patients themselves and it is minimally surgery of the body.Furthermore, in preclinical studies the number of test animals can be reduced (due to the need for lower blood volumes) which is in accordance with the 3R requirement of animal studies (replacement, reduction, refinement). DBS LC-MS/MS has been increasingly employed in clinical study and therapeutic drug monitoring for the analysis of a wide spectrum of drug molecules including antipyretics, antitussives, antimalarials, anticonvulsants, antiretrovirals, immunosuppressants, antiepileptics &Vinca alkaloids and in monitoring drug of abuse.DBS samples could be collected by patients themselves or their guardians,the possibilities of collecting clinical pharmacokinetic samples not only from various in-patients but also from outpatients, especially those from remote areas. It is meaningful when there is a need to monitor drugs, for instance tacrolimus and cyclosporine A, with a narrow therapeutic index, but a wide inter-patient and intrapatient pharmacokinetic variation, DBS samples can be taken without delay whenever a concentration related Side effect appears[8]. The obtained analytical results might lead to a necessary adjustment of dose or dose regimen.

Toxicology

There is some limited and early research onto using this method as a screening test for HIV. Now, some in the forensic area have watched the research advancements in this technology and are investigating& researching on its possible application to the forensic science world. In the pharmaceutical industry, there is also a push to get the FDA to more readily accept it in animal studies as it will reduce the numbers of animals needed for a given study. Thus, it will save a lot of money. The whole idea is sagging the amount of blood volume necessary and save money[9].

Epidemiology studies

Nowadays, DBS method widely used in epidemiological studies e.g.,measurement of cholesterol and triglycerides in surveillance study. Fasting plasma glucose, total cholesterol, high-density lipoprotein–cholesterol, and triglyceride levels were assessed in the study[10].

Clinical chemistry

The primary use of DBS in France is systematic neonatal screening[11]. As blood sampling in newborns is difficult, DBS technology represents a viable alternative. DBS testing was set up in 1978 by the French Association for screening and preventing disabilities in children .Sampling of newborns enables the detection of phenylketonuria, hypothyroidism, adrenal hyperplasia, cystic fibrosis and sickle cell disease (in some areas).A positive result always will be confirmed or denied by further specific tests. Beyond its use for neonatal screening, many clinical analytes (Exogenous Nucleic Acids, Peptides – proteins, Lipids, sugars and small molecules, Xenobiotics, Genomics) can be measured using DBS[12].

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