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Derleme Makalesi /Review Article

Dried Blood Spot (DBS) Technology: Analytical Techniques, and Clinical Applications in Biomedical Research and Healthcare

Dried Blood Spot (DBS) Teknolojisi: Analitik Teknikler ve Biyomedikal Araştırmalar ile Sağlık Hizmetlerindeki Klinik Uygulamaları

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Öz

Kurutulmuş Kan Damlası (DBS), küçük miktarda kanın parmak ucu delinmesi gibi minimal invaziv bir yöntemle toplanmasını sağlayan pratik ve düşük maliyetli bir yaklaşımdır. Bu derlemenin amacı, DBS teknolojisindeki güncel gelişmeleri ve sağlık hizmetlerindeki klinik uygulamalarını kapsamlı biçimde ele almaktır. Çalışmada ayrıca, DBS örneklerinin analizinde kullanılan ileri düzey teknikler, özellikle LC-MS/MS, immünoassayler ve PCR gibi yöntemler tartışılmıştır. DBS uygulamalarında dikkate alınması gereken başlıca unsurlar; hematokrit düzeyinin ölçümleri etkilemesi, örneklerin uzun dönem stabilitesi ve kullanılan kart materyallerindeki farklılıklardır. DBS yöntemi; beslenme biyobelirteçleri, hormon profillemesi, terapötik ilaç izlemi ve HIV, hepatit, SARS-CoV-2 gibi enfeksiyon hastalıklarının sürveyansı gibi pek çok alanda kullanılmaktadır. Son yıllarda geliştirilen dijital mikroakışkan sistemler, tek molekül sayımı ve yeni nesil dizileme yaklaşımları sayesinde DBS, kişiselleştirilmiş ve merkezden bağımsız sağlık hizmeti modellerinin geliştirilmesine katkı sağlamaktadır. Teknik sınırlılıklara rağmen DBS, hem yüksek hem de düşük kaynaklı bölgelerde tanısal süreçlerin iyileştirilmesine olanak sunan umut verici bir yöntemdir. Geliştirilecek yeni stratejiler ve standardizasyon çalışmaları ile DBS'nin tıp alanındaki kullanım potansiyelinin daha da artacağı öngörülmektedir.

Anahtar Kelimeler: Kromatografi, Kurutulmuş Kan Damlası, Endokrinoloji, Enfeksiyon Hastalıkları Tanısı, Yenidoğan Taraması, Terapötik İlaç Takibi.

Abstract

Dried Blood Spot (DBS) is a minimally invasive, practical, and cost-effective method for collecting small volumes of blood through a fingertip puncture or similar technique. The aim of this review is to provide a comprehensive overview of recent advances in DBS technology and its clinical applications in healthcare. In addition, advanced analytical techniques employed for DBS analysis, particularly liquid chromatography–tandem mass spectrometry (LC-MS/MS), immunoassays, and polymerase chain reaction (PCR), are discussed. Key methodological considerations include the impact of hematocrit levels on measurement accuracy, the long-term stability of samples, and differences in the materials used for collection cards. DBS has been applied in diverse areas such as nutritional biomarker assessment, hormone profiling, therapeutic drug monitoring, and surveillance of infectious diseases including HIV, hepatitis, and SARS-CoV-2. With the emergence of innovative approaches such as digital microfluidics, single-molecule counting, and next-generation sequencing, DBS contributes to the development of more personalized and decentralized healthcare models. Despite certain technical limitations, DBS represents a promising tool to improve diagnostic processes in both high- and low-resource settings. Ongoing innovation, new strategies, and standardization efforts are expected to further enhance the clinical utility and global adoption of DBS in modern medicine.

Keywords: Chromatography, Dried Blood Spot, Endocrinology, Infectious Disease Diagnostics, Newborn Screening, Therapeutic Drug Monitoring**.**

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1. INTRODUCTION

A century ago, Ivar Bang[1] developed a peculiar application of dried blood for testing. This method was a complete departure from the conventional and popular techniques known at that time. Forty years later, in 1963, Robert Guthrie[2] introduced dried blood spot (DBS) as a means of screening. His utilization of the DBS technique, along with his unique quest to employ the method to screen intellectually handicapped children, was the newborn screening (NBS)[3].

DBS sampling gives researchers an easy, low-budget way to collect blood. A fingertip prick beats a longer needle if you want something less invasive. Public-health scientists like this method because it pinpoints exposure markers quickly. Field teams in remote, low-resource settings rely on it for that reason. Parents appreciate the technique, too; one small dot of blood is far simpler than coaxing a vein in an infant[4].

Since 1963, when Dr. Robert Guthrie introduced a blood extraction method for NBS to confirm phenylketonuria through the analysis of phenylalanine in blood, countless infants have been screened for metabolic disorders[5]. Since that time, newborn screening has been adopted as a routine in all US hospitals. The subsequent development of an MS/MS technique in the 1990s ushered in a new era in which one analysis could simultaneously detect many biomarkers on extensive panels[4].

DBS is a method for collecting small amounts of blood through a fingertip puncture, with samples dried on specialized cards for later laboratory analysis. The blood is placed on a special paper card consisting of cellulose or polymer material. When this is done, the blood is left to dry. Next, the card with the blood is sent to the laboratory where it is examined. Usually, this method is used in various types of tests and research. DBS requires only a small blood sample for testing[6].

There is increasing use of DBS cards to facilitate global health initiatives; surveillance of disease and the distribution of blood samples have become more accessible than ever before. As opposed to pricking a vein, it is more convenient to draw blood samples into a card. The new approach, especially in areas with inadequate infrastructure, is beneficial[7].

One of the key advantages of DBS sampling is the possibility of at-home collection, eliminating the need for trained phlebotomists and facilitating rapid large-scale sampling. Moreover, DBS cards maintain sample stability at ambient temperatures, reducing reliance on cold-chain logistics for storage and transport — a notable benefit in low-resource or remote settings[8].

There is a demand for the development of a miniaturized, portable, automated, and disposable system designed to facilitate the monitoring of various biochemical markers, such as a point-of-care (POC) system, which can deliver valuable health and environmental informatics[9].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a sensitive method with high specificity. Despite its effectiveness, this method isn't regularly used in clinical labs. The main reasons for this are its complicated process and the fact that it is not automated, which makes it hard for lab technicians to use in everyday work[10, 11].

The introduction of triple quadrupole tandem mass spectrometers in clinical laboratories revolutionized the ability to carry out NBS for multiple diseases in a single multiplexed analysis using DBS. For NBS, MS/MS applications have relied on electrospray ionization to introduce gas-phase analytes from extracted DBS into the MS/MS instrument[12].

With the introduction of LC–MS/MS, it became possible to analyze large numbers of small molecules much more easily and quickly than in the past, and the option to reliably investigate more drugs and metabolites under similar analytical conditions[13, 14].

LC-MS-based metabolomics has revolutionized the study of small molecules and has been applied to the

study of acute and chronic diseases, nutrition, biomarker discovery, microbiology, metabolic/endocrine disorders, and precision medicine, which is a concept to tailor medical treatment based on an individual patient's disease molecular characteristics[15].

DBS is a technique for collecting blood to test for inherited metabolic disorders and has been used since the 1960s. The development of LC–MS/MS technology expanded its use, allowing DBS to analyze drugs with just a small blood sample. Today, this method is applied in various areas: examining harmful substances, monitoring drug effectiveness, diagnosing diseases through drug screening, and checking for illegal drugs. DBS offers many advantages, such as requiring less than 50 microliters of blood, faster analysis due to a more straightforward process, and reduced costs[5].

2. Analytical Techniques Applied to DBS

DBS has become a valuable sampling method. Scientists use advanced methods to study these samples. Each method has its own special advantages. These techniques are good for different testing needs.

2.1. Mass Spectrometry (MS)

Mass spectrometry, particularly in LC-MS/MS mode, has become a very sensitive analytical method for the characterization of DBS samples. This method offers excellent sensitivity and specificity, leading to accurate detection and quantitation of a wide range of analytes (such as therapeutics, metabolites, and other xenobiotic compounds) from just a small volume of blood. In therapeutic drug monitoring (TDM) and pharmacokinetic investigations, LC-MS/MS in combination with DBS has been particularly useful for accurate quantifications of drug concentrations, which are critical for dosing strategies. Furthermore, gas chromatography-mass spectrometry (GC-MS) is still a very important complementary method of choice for volatile substances and compounds needing derivatization, and it was a game-changer for drug screening and forensic toxicology applications[3, 16, 17]. High resolution mass spectrometry (HRMS), like matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS), is also significant, particularly for advanced glycomic analyses of N-glycans. Using DBS samples, this technique can differentiate structural differences and is instrumental in identifying biomarkers[18].

Moreover, DBS has been accepted for the quantification of proteins using multiple reaction monitoring (MRM) of LC-MS/MS techniques because of its sensitivity and specificity. This method overcomes limitations specific to immunoassays[19].

2.2. Immunoassays

DBS samples are compatible with immunoassays, crucial in screening programs and serological studies. For antibody detection, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other viral infection detection, Techniques like enzyme-linked immunosorbent assays (ELISA) and high-throughput immunoassays, such as Roche Elecsys, have demonstrated effectiveness. Immunoassays using DBS provide practical advantages such as easy sample transport and storage, making them particularly suitable for large-scale epidemiological surveys and public health monitoring[20, 21].

An innovative application includes Single-Molecule Counting (SMC), which greatly improves the sensitivity of low-abundance biomarkers. With SMC techniques applied to DBS samples, important clinically relevant proteins such as cardiac troponin I, Prostate-specific antigen (PSA), and C-reactive protein (CRP) can be detected, which broadens their clinical usefulness[22].

In addition, optimized electrochemiluminescent immunoassays for DBS offer the possibility of measuring inflammatory cytokines simultaneously, like IL-6, IL-8, IL-10, and TNF-α. These assays are invaluable in research focusing on chronic inflammation and immune response in nonclinical environments[23].

The DBS-based ELISA modifications for detecting anti-SARS-CoV-2 IgG antibodies have shown high sensitivity and specificity in comparison to validated serum-based assays, emphasizing the versatility of commercially available immunoassay kits adapted to DBS, which can facilitate epidemiological and public health-related research[24].

2.3. Polymerase Chain Reaction (PCR)

Polymerase chain reaction remains one of the most important technologies for the diagnosis of diseases using DBS, especially concerning the detection of viral and bacterial nucleic acids. Quantitative RT-PCR (qRT-PCR) has been optimized for DBS and provides reliable results for detecting some pathogens, such as HCV, although sensitivity may be slightly lower than with plasma samples[25].

Innovative methods like pooled qRT-PCR with DBS show promising results for epidemiological surveillance of malaria. These methods enable collecting and testing samples from entire communities accurately, providing the ability to check malaria often and reliably, even in areas where there are not many healthcare facilities[26].

In addition, PCR tests have significantly improved the ability to diagnose congenital cytomegalovirus (CMV) using DBS. The development of better techniques for extracting DNA has made these tests more sensitive and more useful in medical settings[27].

2.4. Other Analytical Methods

Recent innovations continue to expand DBS applications. Novel paper-based extraction and direct analysis techniques are improving turnaround time and reducing sample processing complexity. Optimized DNA extraction protocols, such as Chelex-100 and TE-buffer-based methods, are enhancing genetic analysis from DBS samples in both clinical and entomological research[22, 28].

3. Clinical Applications for DBS Testing

3.1. Nutritional Status and Vitamin Deficiencies Assessment

DBS has become a dependable way to check nutritional health and diagnose vitamin deficiencies. One of the best things about DBS is that they're easy to collect, transport, and store, which makes them especially useful in remote areas or places that do not have a lot of resources.

3.1.1. Fat-Soluble Vitamins and Carotenoids

DBS combined with LC-MS/MS technology has been successfully validated for analyzing fat-soluble vitamins such as vitamins A and E and carotenoids, including lutein, zeaxanthin, and β-carotene. These methods provide high extraction recoveries and low matrix effects, enabling accurate determination of micronutrient levels within expected physiological ranges. This method offers a non-invasive alternative for monitoring nutritional status and supporting dietary interventions in clinical and community settings[29].

3.1.2. Vitamin A

Recent studies showed that measuring Vitamin A derivatives serum retinol concentrations demonstrated the reproducibility and validity of DBS compared to conventional venous blood sampling using HPLC. DBS offers particular advantages in epidemiological studies of preschool children, where invasive venipuncture may be challenging. Furthermore, the accurate estimation of total body vitamin A stores using retinol isotope dilution (RID) techniques with DBS provides nutritional assessments in low- and middle-income countries[30, 31].

3.1.3. Vitamin D

Vitamin D levels have been successfully measured in DBS samples using LC-MS/MS, offering a robust method even under different environmental conditions. Studies indicate that the variation in temperature and storage conditions doesn't affect quantitation of Vitamin D metabolites such as 25-

hydroxyvitamin D3 (25OHD3) and 3-epi-25OHD3 in DBS, thus this method is an ideal solution for large-scale assessments in geographically dispersed populations. Additionally, DBS assays have demonstrated reliability even after repeated freeze-thaw cycles, reflecting their practicality in community-based studies[32, 33].

3.1.4. B Vitamins

Many early studies included vitamin B development and validation of DBS-based methods, including vitamins B1 (thiamin), B2 (riboflavin), and B6 (pyridoxal phosphate) using LC-MS/MS, which enables accurate quantification of these vitamins. This process simplifies vitamin B assessments compared to traditional ELISA techniques that can be influenced by genetic polymorphism. Also, this DBS method is more practical for pediatric nutritional screening, enabling easier, non-invasive sample collection from children[34].

3.1.5. Folate (Vitamin B9)

Using stable isotope dilution assays linked to LC-MS/MS has improved the measurement of B9 (folate) in DBS. The key marker of folate status is 5-methyltetrahydrofolate assessment in DBS assays, which are highly sensitive and precise and provide reliable folate monitoring in whole blood and plasma spots. These methods solve the limitations associated with folate stability and improve the accuracy of its assessments, which is particularly valuable in large-scale epidemiological surveys and public health screening programs[35].

3.2. Metabolic and Inherited Diseases Screening

DBS is a game changer in the screening and diagnosis of metabolic and inherited diseases, especially in newborn screening. The use of DBS provides early detection and diagnosis for timely intervention and improved patient outcomes.

3.2.1.Newborn Screening (NBS)

Newborn screening in DBS is the most common application used to early identify metabolic disease and genetic disorders, enable timely intervention, and provide effective treatments to prevent severe or irreversible symptoms. The DBS assay is crucial for the diagnosis of metabolic diseases such as PKU, maple syrup urine disease (MSUD), congenital hypothyroidism, and other metabolic errors. The advantages include non-invasive, ease of collection and storage, and suitability for mass screening in diverse populations[36, 37].

3.2.2.Lysosomal Storage Disorders (LSDs)

The screening and monitoring of lysosomal storage disorders (LSDs), such as Gaucher disease, Pompe disease, Fabry disease, and mucopolysaccharidoses (MPS), have been improved by DBS assay. Methods such as fluorometric enzyme assays and MS/MS validated to DBS samples to enable accurate, robust diagnosis, and early treatment intervention, making DBS a reliable choice for LSD screening[37, 38].

3.2.3.Next-Generation Sequencing (NGS)

Recent advancements have combined new sequencing technologies with DBS sampling, improving diagnostic tests. These advanced sequencing methods on DBS enable genomic screening for inherited metabolic disorders. The technology can quickly spot pathogenic mutations, even when only a small DNA sample is available. However, this technology faces challenges such as variable coverage across genomic regions and difficulty reliably detecting insertions/deletions. Despite these limitations, its ability to detect a broad spectrum of genetic mutations supports its future integration into NBS programs, potentially enabling more accurate and comprehensive early diagnosis[39].

3.2.4. Digital Microfluidics (DMF)

Digital microfluidics is a cutting-edge technology that works with DBS to screen newborns for metabolic disorders, such as LSD. DMF uses electronic control to move tiny drops of liquid, which helps conduct enzyme tests quickly, accurately, and affordably. Platforms like SEEKER® have received approval and are already used in NBS programs.

These platforms provide a reliable and scalable solution for widespread NBS[40].

3.2.5. Gaucher Disease Screening

DBS is an invaluable tool for screening and monitoring Gaucher disease. Gaucher disease is a type of LSD caused by glucocerebroside accumulations because of the body's having a glucocerebrosidase deficiency. By early detection of this disease and closely monitoring it with DBS, doctors can manage the condition much better. This enables them to start treatments quickly, such as enzyme replacement therapy, which can reduce health complications and improve overall patient results. This clearly demonstrates the value of DBS tests in managing inherited metabolic disorders effectively[41].

3.3. Infectious Diseases Diagnosis and Monitoring

DBS has greatly improved the diagnosis and monitoring of infectious diseases. They are especially helpful in areas with limited medical facilities or that are hard to reach. DBS makes it easier to detect and track diseases like HIV, hepatitis B (HBV), and HCV, SARS-CoV-2, and other viral infections. This method has made a significant difference in how healthcare is provided in these resource-limited areas.

3.3.1. HIV Diagnosis and Monitoring

DBS technology is a useful tool for diagnosing and monitoring HIV, especially in remote areas or places with limited resources. It allows for sensitive and specific HIV RNA measurement, similar to the traditional method using venous blood. In addition to this, DBS tests can monitor how the immune system responds by examining important immune markers. This offers important insights into how the disease progresses in children with HIV. This approach is particularly valuable in regions where collecting and transporting regular blood samples is difficult, making healthcare delivery more effective in challenging environments[42, 43]. In addition, DBS now offers a way to test for HIV nucleic acids using self-sampling through the internet. This makes testing more accessible and allows individuals to remain anonymous, keeping their information private[41].

3.3.2. Hepatitis B and C.

DBS testing is very effective for diagnosing and monitoring the treatment of HBV and HCV. It accurately finds and measures the virus's genetic material, giving excellent results in diagnosis, which makes it ideal for large-scale screening. DBS testing makes handling samples easier, reduces biohazard risk, and keeps samples safe during transport and storage. It is beneficial in areas with limited healthcare services by making it easier to screen for hepatitis and track treatment progress. This shows great promise in improving access to hepatitis care worldwide[44, 45]. Systematic reviews demonstrate that DBS tests accurately diagnose HBV surface antigen (HBsAg) and HCV antibody (HCV-Ab) with high sensitivity and specificity[46]. In addition, research indicates that DBS offers an affordable and straightforward approach for conducting wide-ranging community hepatitis screening. This technique allows testing programs to expand their reach and include more individuals effectively[47].

3.3.3.SARS-CoV-2

DBS is an effective way to diagnose SARS-CoV-2. They preserve important blood components like leukocytes and biomarkers, essential for studying immune response and gene activity. With DBS samples, healthcare professionals can detect if a person has a SARS-CoV-2 infection by checking for specific biomarkers like CD169 that remain intact in the sample. This method is especially beneficial for monitoring the virus's spread and conducting research because it is stable, easy to transport, and requires only a small blood sample from a finger prick. As a result, SARS-CoV-2 testing becomes more accessible to people in various and remote areas[48].

3.3.4. Cytomegalovirus (CMV)

DBS methods are useful for detecting congenital cytomegalovirus (cCMV) infections. These methods use PCR assays to identify viral DNA. DBS techniques are very sensitive, providing a good, cost-effective, quick option for large-scale population screening. Though there are some challenges with sensitivity compared to saliva or urine tests, DBS is still a practical choice for widespread screening. It supports early detection, allowing for timely treatment to begin[27].

3.3.5. Other Infectious Diseases

DBS samples showed potential for assessing immune responses to diseases like cholera, Enterotoxigenic Escherichia coli (ETEC), and typhoid fever. DBS samples were used to measure antibodies, which help to evaluate how vaccines work and if people have been naturally exposed to these diseases. DBS is also used for diagnosing and identifying genes in severe bacterial infections like Neisseria meningitidis. DBS samples are stable and easy to transport, which is particularly helpful in remote areas where samples move quickly and using regular testing methods can be complex[49, 50]. DBS has been successfully used in prisons. It is helpful for testing and treating infectious diseases. This increases access to healthcare for people who are incarcerated, allowing them to receive necessary medical attention[51].

DBS technology is advancing and enhancing our ability to identify and track infectious diseases. It offers practical and effective solutions for medical and public health purposes globally.

3.4. Therapeutic Drug Monitoring (TDM)

DBS technology is becoming more popular for TDM because it offers several advantages. It is practical and easy to use, and less invasive. DBS can be used with a wide range of medications, such as antiepileptics, antibiotics, antidepressants, antipsychotics, and anticancer drugs. This makes it a flexible tool in monitoring how well these drugs are working in the body.

3.4.1. Antiepileptics

DBS plays an important role in the utility of the therapeutic monitoring of antiepileptic drugs (AEDs), including drugs like carbamazepine, phenobarbital, valproic acid, and phenytoin, and their metabolites. The use of LC-MS/MS enables us to measure drug levels with great accuracy, like traditional plasma tests. Research has shown that AEDs remain stable in DBS samples under different storage conditions. This stability ensures DBS is a reliable method, especially in remote locations or places with limited medical resources[52, 53].

3.4.2. Antibiotics and Anti-infectives

DBS is a method used to monitor different medications, including antibiotics, antifungals, and antiretrovirals. This approach substantially improves adherence monitoring and facilitates individualized dosing, which is particularly beneficial in outpatient and resource-limited environments. Research shows that DBS can accurately measure medications like voriconazole, which have individual pharmacokinetic variability. This method offers quick results and easy sample collection, making it ideal for critically ill or outpatient populations[54, 55].

3.4.3. Anticancer Drugs

DBS is valid for therapeutic monitoring of anticancer drugs. These drugs frequently have a very narrow. The consensus range is that it is safe and effective, and each person may process these drugs differently. There was the initial concern of the dose relationship with DBS and plasma levels. Fortunately, these refined conversion techniques now accurately convert DBS levels into blood levels that may be estimated more precisely. This upgrade will also greatly help patients needing to supply a sample. Clinicians can thus more closely monitor doses to the needs of individual patients, enhancing the effectiveness of cancer treatments[56].

3.4.4. Psychiatric Drugs

DBS sampling has made monitoring psychiatric medications easier, such as antidepressants,

clozapine, mood stabilizers, and serotonin reuptake inhibitors. The DBS method makes patients more comfortable and helps them stick to their medication plans. Especially for psychiatric patients sensitive to invasive sampling techniques. Robust analytical and clinical validation shows that DBS is reliable for regular use in mental health care. Home-Sampling also supports regular checks and helps patients keep up with their treatment when not in a hospital or clinic setting[57, 58].

3.4.5. Methodological Considerations and Clinical Validation

To effectively use DBS in healthcare, it's crucial to focus on important aspects like the type of DBS card, variations in hematocrit (HCT) levels, and comprehensive analytical validation. HCT levels are key because they impact the homogeneity and accuracy of the DBS sample. Extensive testing has shown that drug concentrations measured by DBS match closely with those found in plasma, demonstrating that DBS methods are effective in clinical settings. Guidelines from the International Association for TDM and Clinical Toxicology (IATDMCT) emphasize the need for careful validation. This includes detailed analytical and clinical testing, as well as careful handling and storage of samples[59-62].

3.4.6. Innovations and Drug Response Monitoring

Thanks to recent advancements in analytical automation, the process of DBS analysis is now much more efficient, reliable, faster, and with reduced analytical variability. Currently, DBS plays a vital role in Drug Response Monitoring (DRM). It can observe biomarkers that indicate therapeutic efficacy or adverse drug reactions. This ability helps doctors to track treatments specifically for each person by linking how the body processes a drug with the drug's actual effects. This approach supports doctors in making better decisions for their patients, which leads to improved health results[63, 64].

3.5. DBS and Endocrinology

DBS sampling is a useful technique in the field of endocrinology. It provides minimally invasive, convenient, and reliable measurements of various hormones. It is used in both medical settings for diagnosing patients and in scientific research applications.

3.5.1. Steroid Hormone Profiling

Steroid hormone profiling using DBS sampling by LC/MS-MS is an easier way to analyze hormone levels. It allows for reliable measurement of key steroid hormones such as testosterone, androstenedione, progesterone, cortisol, and corticosterone. These hormones are checked using small blood samples from a simple finger prick. DBS profiling is especially helpful in medical and research studies that need to examine several hormones at the same time. It offers accurate and sensitive results comparable to those from traditional blood tests taken from veins, making it a valuable tool in evaluating hormone interactions and functions[65, 66]. DBS is beneficial for measuring testosterone in people with hormone issues like hypogonadism and polycystic ovary syndrome (PCOS). Additionally, they are useful for monitoring treatments like testosterone replacement therapy, providing a less invasive and more practical method[67, 68].

3.5.2. Clinical Applications and Stability

DBS techniques are efficient for testing hormones in specific health issues. These are important for conditions like congenital adrenal hyperplasia (CAH), adrenal insufficiency, and hypogonadism, where frequent hormone monitoring is required. The hormones in DBS stay stable and reliable, even when stored in different conditions. Many tests have proven this stability, even if temperatures vary during transport and storage. Hormones such as testosterone and cortisol can remain stable for several months. This makes them very useful for medical research and for studies that track health in groups of people over a long time[65, 69].

3.5.3. Methodological Considerations

DBS has many important benefits, but handling some scientific aspects for accurate hormone measurement is essential. One key issue is the effect of HCT, or the amount of red blood cells in the blood. This can influence measurement results, as can the substances around the blood. Adjustments for HCT or using standard methods to measure blood volume are important to keep tests precise. Additionally, Extensive method validation is required to ensure they are accurate, reliable, and consistent, which is crucial when these tests are used in clinical and research applications[67].

4. CONCLUSION

DBS technology is now a widely used tool in many areas of healthcare and science. Its successful integration into NBS, TDM, infectious disease diagnostics, endocrinology (hormone studies), and nutritional assessments highlights its broad clinical utility. This shows DBS's significant impact on clinical practice and scientific research. The benefits of DBS are that it is minimally invasive, cost-effective, easy to handle, and has stable samples, making it especially useful in places with limited resources and large health studies involving many people.

Advances in technology for analyzing samples, particularly through LC-MS/MS and HRMS, have greatly increased the variety of substances that can be measured from DBS. These technologies offer sensitive and precise measurement of drugs, hormones, metabolites, and genetic material. New approaches like DMF, single-molecule immunoassays, and NGS are continually enhancing the capabilities of DBS. They are paving the way for new possibilities in precision medicine, monitoring public health, and diagnosing medical conditions from remote locations.

DBS has some limitations despite its successes. Major challenges include changes caused by HCT levels, uneven spot distribution, and differences in the sample compared to plasma or serum. It is important to validate these methods to ensure analytical equivalence carefully. DBS has a lot of potential for healthcare that is more widespread and less centralized. Still, pre-analytical and analytical protocols are standardized before they can be used more widely in clinics. Moving DBS methods from research settings to standard clinical practice often requires a thorough check for their diagnostic accuracy, reproducibility, and long-term stability.

Future research should standardize collection methods, improve the accuracy of measuring sample volumes, and develop automated systems that integrate DBS easily into clinical workflow. Another promising area is expanding the use of DBS for continuous monitoring, at-home sampling, and quick treatment adjustments. Collaboration among clinicians, chemists, laboratorians, and regulators is essential to maximize DBS's potential. DBS is a powerful tool in modern medical tests, offering simple and scalable solutions to health challenges worldwide. With ongoing innovation and careful validation, DBS is set to be a key player in the future of personalized and decentralized healthcare.

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