

■ REVIEW ARTICLE

Dried Blood Spot Testing "A game changer" in Anti-doping strategies: A Review

¹Adil Ali Ansari, ²Aman Sachdeva

ABSTRACT

BACKGROUND: Dried blood spots (DBS) have been used for years as a supplementary matrix in sports drug testing. Dried Blood Spots are a promising technique for minimally invasive sample collection in a variety of analytical disciplines, such as therapeutic drug monitoring, preclinical drug development, and diagnostic investigation of metabolic abnormalities in newborns. The increasing potential of DBS has been highlighted in the scientific literature, particularly when it comes to drugs prohibited in world sports. World Anti-Doping Agency (WADA) has planned to incorporate DBS as a new and much more efficient way of assessing the athletes for any prohibited ergogenic aid in upcoming Tokyo Olympics 2021. This literature seeks to evaluate the scope of effectiveness of the DBS method in identification of various banned drugs by examining previous data derived from literatures on DBS. Based on the researches analyzed, it is concluded that DBS methods is very much implementable in identification of variety of ergogenic aids along with being much more efficient way of identification.

KEY MESSAGE: DBS method of blood doping analysis can prove to be a new and time saving process of doping analysis in the upcoming Tokyo Olympics 2021 due to its vast applications & cost effectiveness and considerable safety during COVID-19.

KEYWORDS | dry blood spots, dbs technique, doping, ergogenic aids, tokyo olympics 2020

Author's Credentials:

¹Assistant Professor, Department of Physiotherapy, Sharda University, Greater Noida 244001, India.

²Head & Consultant, Department of Physiotherapy, Sitaram Bhartiya Institute of Science & Research, New Delhi 110016, India.

Corresponding Author:

Adil Ali Ansari, Assistant Professor, Department of Physiotherapy, Sharda University, Greater Noida 244001, India.

Email:

adil.ali@sharda.ac.in



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INTRODUCTION

FOR MANY YEARS, DRIED BLOOD SPOTS (DBS) has been used as a supplementary matrix in sports drug testing. Dried Blood Spots (DBS) are a promising technique for minimally invasive sample collection in a variety of analytical disciplines, such as therapeutic drug monitoring, preclinical drug development, and diagnostic investigation of metabolic abnormalities in newborns. DBS sampling is distinguished by its cost-effectiveness, simplicity, resilience, and ease of storage and shipment which is a need of this hour during this pandemic.

An increasing potential of DBS has been highlighted in the scientific literature, particularly when it comes to chemicals prohibited in competition. World Anti-Doping

Agency (WADA) has planned to incorporate DBS as a more efficient way of assessing the athletes for any prohibited ergogenic aid in upcoming Tokyo Olympics 2021. The WADA Prohibited List has eleven classes of prohibited substances and three classes of illegal methods, with four classes (stimulants, narcotics, cannabinoids, and glucocorticoids) regarded to be prohibited during competition.

If the analysis reveals blood concentrations of the prohibited substance below recommended or accepted levels, indicating an impact on the athlete at the time of competition, the result managing authority (RMA) obtains crucial information for a more personalized case management process that takes into account the additional evidence in

the athlete's favor. The intrusive and costly collection and transport of blood samples is a major restriction in that scenario. Dried blood spot (DBS) matrices, which are easily made, relatively inexpensive, and have stabilizing properties for the target analyte, are a possible option in this context. In conjunction with new advancements in sampling techniques, (semi-)automated DBS sample preparation alternatives, and significantly improved instrumental responsiveness in bioanalysis, collecting matched pairs of urine and DBS samples in regular doping controls would provide a valuable contribution for the overall picture of the testing. This literature seeks to evaluate the scope of effectiveness of the DBS method in identification of various banned drugs by examining previous data derived from literatures on DBS.

METHOD

A literature search was conducted across PubMed and Science Direct databases, including the reference lists of relevant papers which ranged in duration of 2015 to 2021. The specific terms used for identifying relevant literatures were 'Dry Blood Spot', 'DBS technique', 'Doping', 'Anti-Doping', 'ergogenic aids', 'athletes', 'Tokyo Olympics'. Reference lists of articles obtained from this search were also examined for additional relevant articles. The inclusion/exclusion criteria for studies were based on their potential relevance to the effectiveness of DBS methods in identification of ergogenic aids.

In a study conducted by Kim *et al.*, (2015) in 453 DBS samples, development and validation of an LC-MS/MS (Liquid Chromatography – Tandem Mass Spectrometry) method was done to calculate the reference intervals of cortisol, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, and rostanedione, corticosterone, and 11-deoxycorticosterone where the samples were taken from Korean people of various ages.¹ At three concentrations, the accuracy, precision, matrix effects, and extraction recovery were all good. The linearity

range for cortisol was 1-100 ng/mL and 0.5-50 ng/mL for other hormones, indicating that the LC-MS/MS method and reference intervals validated in the Korean population may be used to assess seven drugs in DBS. In a study conducted by Tretzel *et al.*, (2014) using DBS, eight anabolic steroid esters (nandrolone phenylpropionate, trenbolone enanthate, testosterone acetate, testosterone cypionate, testosterone isocaproate, testosterone phenylpropionate, testosterone decanoate, and testosterone undecanoate) as well as nandrolone were studied.² It was concluded that DBS can be used to analyze anabolic steroid esters in doping controls, potentially simplifying the confirmation of exogenous testosterone administration.

Similarly, in a study conducted by Peng *et al.*, (2000) an oral 120-mg dosage of testosterone undecanoate, collection of dried blood spots and plasma from six healthy Caucasian participants was done.³ Gas chromatography–mass spectrometry was used to assess nonconjugate testosterone, testosterone glucuronide (TG), androsterone glucuronide (AG), and etiocholanolone glucuronide (EtG). The results on dried blood spots and plasma were very similar. The testosterone glucuronide/testosterone ratio in blood or plasma was found to be a sensitive and specific marker for oral testosterone undecanoate (TU) intake (significantly increased for up to 8 hours after intake; $P < 0.05$), but not for intramuscular testosterone propionate and testosterone enanthate administration.

Apart from anabolic steroids, in a comparative study done by Kojima *et al.*, [2016] compares a quantitative laboratory urine assay to a liquid chromatography-tandem mass spectrometric approach for detecting ephedrine and methylephedrine utilizing dried blood spot testing.⁴ At 4-10 hours after ephedrine administration, the urine concentration of ephedrine did not surpass the threshold in two patients. The maximum levels of ephedrine and methylephedrine in the blood were attained 2-8 hours after ingestion. The blood concentrations

had a low inter-individual variability, and the findings revealed that urine pH and/or urine volume can have a big impact on ephedrine and methylephedrine excretion.

As the banning of various growth hormones in the WADA prohibited list is concerned, in a study conducted by Reverter *et al.*, [2016] a clinical trial was done with healthy volunteers who were given a low subcutaneous dose of recombinant human growth hormone (0.027 mg-1kg-1day-1person-1) for three days.⁵ A comparison was made between finger prick DBS and paired time serum samples from arm venepuncture and concluded that the DBS-based protocol's analysis revealed that positive growth hormone misuse may be detected with just a single blood spot. The detection window for DBS was confirmed in all examined samples up to 8 hours after administration, and in half of the instances it was extended to 12 hours. For 12 hours following injection, serum positivity was detected in all of the samples examined.

Apart from sensitivity to prohibited drugs, the DBS method has also shown reliable results in detection of autologous blood transfusion doping method which is used by athletes in enhancement of red blood cell counts and thereby increasing performance. A study was done by Cox *et al.*, (2017) on detection of autologous blood transfusions via novel dried blood spot method, autologous transfusion of 15 subjects who received blood and 11 subjects who received saline.⁶ After transfusion, the average CD71/Band3 ratio (immature reticulocytes (IRC) and red blood cells (RBC)) in the blood group was measured from the saline group at days 5, 6, 13, and 20. (Analysis was done via cell-specific proteins digested with trypsin and measured by mass spectrometry). It was found that the average CD71/Band3 ratio decreased to a minimum of $61 \pm 8\%$ of baseline. Based on experimentally defined criteria, the CD71/Band3 ratio could detect 7 out of 10 blood transfusion subjects. Thus, concluded that the DBS method could improve detection of autologous transfusion.

CONCLUSION

DBS method of blood doping analysis can prove to be a new and time-saving process of doping analysis in the upcoming Tokyo Olympics 2021 due to its vast applications and cost effectiveness. Considering the pandemic situation of COVID-19, the DBS method can prove to be minimally invasive and least infectious method with high and significantly suitable measurements of results. On the same aspects, the rise of new doping methods and substances should be considered and more researches in DBS effectiveness are need of the hour for the same. **IJFMP**

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