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Abstract Topic:- Clinical Genetics

Abstract Title:- A Novel Method of HPLC using DBS for Hemoglobin Variant Analysis and its Comparison Between Conventional High Performance Liquid Chromatography (HPLC) using Whole Blood Vs Dried Blood Spot (DBS) – HPLC

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Aims:-HPLC on whole blood samples is considered gold standard method for screening & diagnosis of Hemoglobinopathies. Collection of whole blood and temperature-controlled logistics to a laboratory, limits its use in population screening especially in remote, tribal regions for Sickle-cell, Beta-thalassemia and other variant Hemoglobinopathies. The present study was aimed at comparing method developed for analysis of DBS collected from finger prick for all age groups on a high throughput open HPLC system against whole blood samples analyzed by the conventional HPLC system.

Methods:- The study compared of total 202 samples (age 1-95 years) from HPLC analysis of Hemoglobin (Hb) variants on DBS stored and transported at ambient temperatures to laboratory at 3, 5, 11, and 24, post collection, to those analyzed from fresh whole blood collected

and analyzed on the same day on HPLC. The Hemoglobinopathy status of the patients were unknown at the time of collection.

Results:- The two-methods showed excellent correlation and agreement for all Hemoglobin fractions, when each Hb Fraction percentage value from both WB-HPC and DBS- HPC was

individually statistically compared. Bland-Altman plot were also drawn for limit of agreement (LoA) for each Hemoglobin Fraction which showed good agreement between the two methods, across all clinically important percentage ranges i.e., from 0-50% for HbF, from 0.5-90% for HbA, from 0.5-10% for HbA2, 10-90% for HbE and 10-80% of HbS.

Conclusions:- The DBS- HPLC method is found an excellent choice for unified, integrated, and comprehensive screening which can ease the process of collection, decrease the time and resources utilized and save substantial time & cost for screening operations for all age groups. The method allowed accurate interpretation of all major Hemoglobinopathies, and thus can be used in resource poor, remote and geographically vast regions in India.

Keywords:- Dried Blood spot-High Performance Liquid Chromatography (DBS-HPLC), Sickle-cell, Beta-thalassemia, Population screening