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## RESEARCH ARTICLE

### Comparison of an alternative and existing dbs matrix for estimation of thyroid stimulating hormone (tsh) in adults.

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#### Abstract

Currently, Whatman 903 (W-903) filter paper is the only filter paper that has been used for collection of blood samples in thyroid hormones. This commercially available filter paper has so expensive which is not suitable for epidemiological study conducted in developing countries. We therefore introduced a new matrix for collection of blood samples and also compared with commercial available matrix, Whatman filter paper (W-903). We used two matrices initially for standardisation purpose viz., the conventional Whatman dbs cards (#903 10531018) & pre coated TLC silica gel matrix (60F254, Merck 20x20 Cm), the latter with cost-effectiveness in view. Both were subjected to validation for optimum working conditions. The assay characteristics for Whatmann DBS card and TLC matrix were found to be as follows: correlation coefficient 0.982 & 0.976, slope 0.070 & 0.068, coefficient of variation 0.32-0.77% & 0.40-0.85%, sensitivity 0.0287 & 0.0286 mIU/L and interassay variation 0.40% & 0.50 respectively. The assay characteristics of the calibration curve for FT4: correlation coefficient 0.926, slope 0.094, coefficient of variation 0.84-4.12% and inter assay variation 3.03% for TLC silica matrix. Drying time (0.5, 1.5, 2.5, 3.5, 4.5, 5.5 h), elution time (1.0, 3.0, 5.0-16 h), percent recovery (83-98%) and linearity at varying dilutions were determined. Stability studies for TSH samples were conducted at variable temperatures and half life found to be temperature dependent viz., 24.54 days, RT; 180.45, 4°C; 206.25 days, -20°C. Owing to its cost-effectiveness and comparable results, TLC silica matrix was preferred for subsequent studies under optimized conditions.

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#### Introduction

Dried blood spot sampling technique has emerged as a pertinent method for both qualitative & quantitative bioanalysis context (Sharma et al., 2014). DBS has been mainly used for the neonatal screening (Pitt 2010), therapeutic drug monitoring (Edelbroek et al., 2009, Jantos 2013), preclinical (PK/TK), clinical study, metabolomic (Yin et al., 2015), disease diagnosis (Jantos 2013), detection of inborn errors of metabolism, lipidomics (Koulman et al., 2014), thyroid hormone screening in new born babies (Miyai et al., 1978, Mizuta et al., 1982, Torresani et al., 1986, Lemonier et al., 1999, Bellisario et al., 2000, Gruneiro-Papendieck et al., 2000, Zimmermann et al., 2006, Najafi et al., 2008, Boemer et al., 2009, Adachi et al., 2012) and phenylalanine estimation in newborns phenylketonurea patients (Guthrie and Susi 1963). Only recently has the technique been considered for screening in adults in two studies only (Hoffman et al., 2003 & 2013).

Many studies have demonstrated the use of Whatman's FTA Paper for the estimation of thyroid stimulating hormone in newborns (Ref). Several modified DBS cards with improved extraction recovery versions are currently in use which includes alginate foam, on line DBS, DESI & DART System, Hema spot & BSD robotics. Until now, an alternative

DBS matrix has not been available for laboratories to utilize throughout their various stages of processing, collection through sample processing.

This method is a sampling method for blood samples on filter paper. After initial sampling filter paper is dried, stored and taken to the laboratory for further analysis. DBS method offers distinct advantages over conventional serum sampling technique e.g use of small blood sample, ease of collection, reduced sample processing & shipping cost and various associated & ethical benefits.

Uses of commercially available DBS matrix become more costly for epidemiological surveys. In this present study a TSH levels of an adult population have been determined using novel DBS sampling method with slight modification.

## **Material & Method:-**

### **Materials:-**

Matrix used were purchased from G E healthcare Pvt ltd (whatman 903 dbs card), Gurgaon & MM Biotech Pvt ltd (TLC Silica) New Delhi. Thyroid stimulating hormone (TSH) standards (0 to 40 mIU/ml) were purchased from cal biotech ltd, New Delhi. Disodium hydrogen phosphate, potassium di hydrogen phosphate from ficher scientific, sodium hydroxide from SRL and sodium chloride from merck were purchased. ELISA spectrophotometer & Immunowasher were purchased from BIORAD and pH meter was supplied by Chandra scientific systems Pvt Ltd. All blood samples for TSH analysis were collected from the volunteers with mixed age groups. Hormone free Serum was prepared in laboratory by activated charcoal method & washed erythrocytes were separated from blood pool.

### **Methods:-**

Finger pricked method was used to collect blood sample (McDade 2014). Blood was applied to matrix & dried for different times. The responses were monitored by punching out 4 mm diameter spot at 0.5, 1.5, 2.5, 3.5, 4.5 & 5.5 hr intervals. These dried blood spots were eluted with different elution solvents for different incubation time. i.e millipore water, distill water & phosphate buffer saline at pH 7.4. The responses were observed using ELISA spectrophotometer. The phosphate buffer saline (Xianing et al., 2007) was prepared in millipore water & distills water. The natural pH of distill water (ph 6.8) and millipore water (pH 6.1) were adjusted to 7.4 & measured the responses. Different molar phosphate buffer saline was also prepared and measured responses.

TSH was assessed in Dry Blood Spots using a modification of Zimmerman et al., 2003 and read in ELISA plate reader (Adachi et al., 2012). Pre coated TLC silica gel matrix (60 F254 20 x 20 cm) was used for blood collection, instead of Whatman paper. In order to minimize matrix differences and maximize comparability between calibrators and unknowns, DBS standards was prepared (Mcdade 2014). Each concentration of TSH standards was then added to an equal volume of washed erythrocytes (1:2 dilutions). Mix gently with inversion for 5 min, then applied each calibrator to matrix. Blood samples were also spotted on whatman dbs card and TLC silica matrix. After drying of 3.30 hr, punched out a disc of 4 mm diameter and placed in appendorf tube. Elution solvent (110  $\mu$ l) added to each tube, intermittent vortex for 1 mint and incubated for 5 hrs at 4-6<sup>o</sup>C. 50  $\mu$ l of standard elute and sample elute added to anti-TSH antibodies coated micro wells and were incubated with peroxidase labeled anti-TSH monoclonal antibodies for 60 min at room temperature. After three time washing, the unbound antibodies were washed off and bound conjugate remains in micro well. These bound conjugates further react with substrate 3,3',5,5'-tetramethylbenzidine (TMB), incubated and added stop solution to each well. Read absorbance on ELISA reader at 450 nm within 15 minutes after adding the stopping solution. By comparing the OD of the test samples to this standard curve, the concentration of the TSH was determined.

Four standard curves were run on different days. Standards and controls were run in set of five. Figure depicts a standard curve obtained with TSH standard dried blood discs. The measurable range was 0.25 to 20 mIU/ml. The smallest amount detectable was 0.0287 and 0.0286 mIU/ml on whatman dbs card and TLC silica matrix from the pint where the 95% confidence limits of the response at zero doses intersects the standard curve. The intrassay variation for each run & interassay variation were calculated.

Linearity of the assay was also measured by using TSH standards with low, medium & high conc. (0.25, 2.5, 20 mIU/L) were diluted with equal amount of washed erythrocytes (300 $\mu$ l) to prepared artificial blood. This artificial blood with known concentration of analyte further diluted with hormone free serum (1:1.5, 1.3). Then spotted on the both the matrix (Whatman & TLC). Calculated the ratio of observed to expected analyte concentration was used as measure of accuracy.

Effect of hematocrit was assessed by preparing artificial blood with known concentration of TSH varying hematocrit (25% to 75%). Human K2EDTA whole blood was centrifuged at 4 °C for 10 min at 1000 g. The resulting plasma & erythrocytes fractions were separated as Mac dade 2014. Hormone free serum was prepared by added 1gm norit A Charcoal to 19 ml of serum. For the preparation 30 % hematocrit, 30 µl of RBC mixed with 10 µl TSH concentrations from each calibrator and 60 µl of hormone free serum was added to make up the volume of 100 µl. Same protocol was followed for preparation of different hematocrit with changing the amount of RBC, hormone free serum and known amount of TSH remain constant at different concentration. Punched out 4 mm diameter disc & measured the TSH response through immunoassay. Recovery was also calculated.

The quality control samples were spotted on whatman filter paper and TLC silica matrix. These matrixes were air dried for 3.30 hr then processed for TSH assay in five replicates from each set. The mean value of TSH concentration was calculated. This amount of TSH was considered as zero day concentration. A set of controls were stored at three different storage condition i.e room temperature, 4°C and -20 °C in air tight zip lock plastic bags with silica balls. The silica balls were regularly checked & changed if required. Controls stored under these conditions were assessed on subsequent hrs 0, 24, 96, 216, 456, 696 for TSH. Percentage of hormone degradation was calculated & half life in all sets was also determined. We used to determine one way analysis of variance to determine the any change in assay values for control stored at different condition for different hours.

#### **Comparison of DBS and Serum:-**

Blood samples were collected from subject (n=20), applied to matrix and also separated serum from similar blood sample. After drying of 3.30 hr, punched out a disc of 4 mm diameter and placed in appendorf tube. Elution solvent added to each tube, vortex and incubated for 5 hrs. DBS elute and serum added to anti-TSH antibodies coated micro wells and were incubated with peroxidase labeled anti-TSH monoclonal antibodies. After washing, the unbound antibodies were washed off and bound conjugate remains in micro well. These bound conjugates further react with substrate 3,3',5,5'-tetramethylbenzidine (TMB), incubated and added stop solution to each well. Read absorbance on ELISA reader at 450 nm within 15 minutes after adding the stopping solution. By comparing the OD of the DBS samples to standard curve with slope 0.068, the concentration of the TSH was determined. Similarly OD of serum sample was compare with standard curve (Slope=0.092) and TSH concentration was estimated.

The correlation coefficient for the two values of same sample (TSH in serum & DBS) was 0.95, (Pearson  $r=0.97$ ) the slope was 1.013 and the intercept was 0.256. Correlation between TSH DBS and TSH plasma concentrations supports the use of DBS for hormonal studies.

#### **Result & Discussion:-**

Dried blood spot a convenient sampling method used for collection of whole blood by heel prick method. This method is useful in resource limited area especially in the developing countries where handling samples for analysis reduced cost as well as time (Brambilla et al., 2003). DBS method required minimal training of personnel with less instrumentation & no risk of injuries (Parker and Cubitt 1999). Many studies have demonstrated the use of Whatman's FTA Paper for the estimation of thyroid stimulating hormone in newborns (Nagataki et al., 1980, Bellisario et al., 2000). Only recently has the technique been considered for screening in adults (Hoffman et al., 2003& 2013). Conventionally cellulose and sometimes non-cellulose based spot cards are used to prepare dried blood spot (Meesters et al., 2012). FDA under 21 CFR 862.1675 has been approved ahlstrom 226-K062932, whatman 903 and perkin almer 226 (CDC, 2009) as DBS cards for sample collection. These cards are manufactured from 100 % cotton liners have no wet strength, spreading, degradation & stability (alginate foam), cost (100 samples approx 7K). Several modified DBS cards with improved extraction recovery versions are currently in use which includes alginate foam, on line DBS, DESI & DART System, Hema spot & BSD robotics. Wilson, S.R.H., 2012 used alginate foam as sampling medium for DBS, despite foam is stable at physiological pH but it disintegrates in the lower pH-range. A new concept, called on-line desorption of dried blood spots (on-line DBS) has been designed to facilitate its implementation into existing laboratory systems by coupling of DBS with liquid chromatography mass spectrometry device (LC/MS) where only DBS is routinely used (Deglon et al., 2009). This method requires high instrumentation laboratories and highly expensive per sample. Hema Spot collection device is a single use, highly expensive (Kit capacity 12 samples @ \$ 99) bar coded device allowing the collection, preservation and storage of blood in one device (www.spotonscience.com). More importantly, DESI & DART System has disadvantage of poor sensitivity due to ion suppression and possible interference from decomposition of metabolites. John Dinan (BSD Robotics) brought the DBS advantage into laboratory by introducing BSD robotic units BSD 600, BSD 600 (Dute) and BAD 700 for uniform puncturing of DBS (Abbott et al., 2010).

The present study being describes here compare the three matrix, two alternative new DBS matrix and one commercial DBS card. The matrixes were compared for physical characteristics of DBS (spot areas) at different hematocrit and accurate quantification of analytes (recoveries). The colour of the blood spot deepens from bright red to deep red when the hematocrit level is increased from 30 to 75%. As the blood hematocrit level changes, the viscosity of the blood changes, high hematocrit levels being most viscous due to the increase in number of blood cells. The variability in viscosity leads to differences in diffusion properties of blood through different matrix, which can directly impact size of the blood spot formed.

**Table 1:-** Comparison of physical characteristics and quantification of three DBS matrix

Matrix Used	HCT(%)c	Diameter (mm)	Radius(mm)	Area(mm <sup>2</sup> )	%Difference from 45% hct	Volume of blood in 4 mm punch(μl)	Recovery (%)
TLC silica matrix	30	7.75±0.478	3.87±0.23	47.69±5.98	23.99	15.65±0.92	87
	45	7	3.5	38.46±0.0	0	17.14	89
	60	6.250±0.25	3.12±0.125	30.81±2.55	-19.89	19.29±0.71	91.2
	75	5.87±0.125	2.93±0.06	27.13±1.12	-26.45	20.46±0.45	92.4
Whatman filter paper	30	14.84±0.10	7.42±0.05	172.9±2.38	34.34	8.08±0.056	77
	45	12.8±0.12	6.40±0.06	128.7±2.45	0	9.37±0.09	79
	60	12	6	113	-12.19	10	79
	75	11.68±0.096	5.84±0.04	107±1.78	-16.86	10.28±0.08	80
Whatman DBS 903	30	10.25±0.25	5.12±0.125	82.62±4.12	7.73	11.73±0.27	86
	45	9.87±0.125	4.93±0.062	76.69±1.91	0	12.16±0.15	89
	60	9.5±0.288	4.75±0.144	71.04±4.30	-7.36	12.67±0.38	91.6
	75	9.37±0.37	4.68±0.18	69.33±5.74	-9.59	12.86±0.47	92.4

Table 1 shows area of the spotted blood decreases going from low to high hematocrit in all matrixes. More viscous blood at higher hematocrit spreads less on all dbs card giving rise to an inverse relationship with hematocrit and spot area. The present study result has been found in agreement with another published study (Denniff and Spooner 2010, Arora et al., 2011, Hudson et al., 2011, Arora et al., 2013).

The are covered by same amount of blood on whatman filter paper was almost more than three times to the TLC silica matrix and two times to the whatman dbs card. More blood spread to the whatman filter paper and also covers more area as compared to other dbs matrixes. Figure 1 illustrate dependence of the spot diameter (mm) on the hematocrit value (%) on TLC matrix (A), whatman filter paper (B) and whatman dbs card.

The volume of blood in 4 mm punch was 50% higher on TLC matrix than whatman filter paper at all hematocrit as shown in table 1. This shows that more analytes is extracted, resulting increase sensitivity or better responses and higher recovery. The recoveries of TSH hormone on the TLC matrix are high as compared to whatman filter paper at each hematocrit level as shown in table 1. The whatman filter paper shows lesser recoveries (79%) at different hematocrit as compared to TLC silica matrix (90%) and commercially available whatman dbs 903 card (90%).

The volume taken from the 4 mm punch is determined by the ratio of the area of the area of the punch to the area of the spot. The blood volume in a 4-mm disc taken from a DBS was positively correlated with hematocrit, over the range 30–75 % hematocrit in all matrixes as found in other published study (Adam et al., 2000, Fan et al., 2012, Lee et al., 2012). As the hematocrit of the spotted blood increased, the amount of blood in the punch also increased, present study represented opposite finding as that of Joanne V. Mei et al 2001. It was apparent from our results that as the percent hematocrit increases, there are approximately a 23%,9% and 21% increase in the perceived sample volume in 4mm punch from 25% hematocrit to 75% hematocrit on TLC matrix, whatman dbs card and whatman filter paper. Similar observations were noticed earlier by O'Broin 1993, Miller et al., 2013.

Samples collected on TLC silica matrix shows almost twice half life as compared to whatman filter paper at various storage conditions (Table 2).

**Table 2:-** Stability study of samples on TLC matrix and DBS matrix

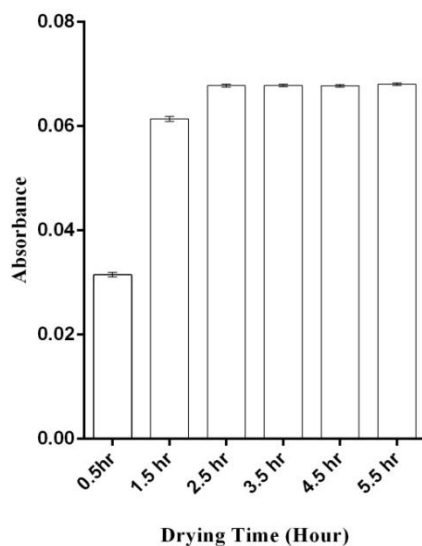
Matrix	At Room Temp			At 4 <sup>o</sup> C		At -20 <sup>o</sup> C		
	Half Life (hr)	Decay Constant (hr <sup>-1</sup> )	Constant	Half Life (hr)	Decay Constant (hr <sup>-1</sup> )	Half Life (hr)	Decay Constant (hr <sup>-1</sup> )	Constant
TLC matrix	577.5	0.0012		3465	0.0002	6930	0.0001	
Whatman filter paper	385	0.0018		2310	0.0003	2310	0.0003	

The half life of TSH were 385, 2310, 2310 days and 577.7, 3465, 6930 days on whatman filter paper & TLC silica matrix at room temp, 40C & -200C respectively. The samples were more stable at TLC silica matrix shows less degradation at different storage condition as compared to whatman filter paper. It has been published that TSH concentration are more stable at room temperature for a week and for a month at 4 °C (Walte et al., 1987) on whatman card 903, present study also having the similar observations in similar conditions. TSH concentration decline if samples are stored at room temperature for more than six weeks, but are stable at -20°C for up to nine months on whatman dbs card (from literature). Similar trend was also observed in TLC silica matrix for sample stability. Ezzi et al., 2010 reported that TSH stable for 2.7 years at room temperature and up to 6.5 year and 4.1 years, when stored at -20°C & 4°C under continuous power supply on whatman dbs card. Mannistor et al., 2007, TSH remain stable for 23 years at -25°C on whatman dbs 903 card.

Whatmann filter paper having more spreading, lesser recovery & less sample stability hence this matrix is not further recommended for the sample collection.

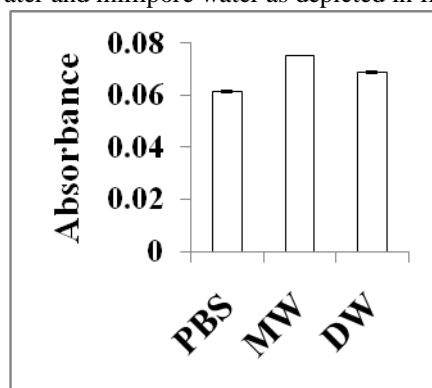
### Assay Protocol:-

Modified DBS protocol produces valid results we need to demonstrate a reasonable level of accuracy, precision, and reliability in our laboratory measurements. It is therefore essential to identify sources of error, to minimize their impact to the extent possible, and validate each parameter for the analyte. In addition, it is important to consider the range of values that can be quantified, and the level of accuracy, precision, and reliability across this range. DBS sampling involves spotting of blood on the matrix, drying, incubation and biochemical assay. Drying may alter the biochemical structure of a molecule and efficiency of the analytes (McDade 2007). Complete drying can be seen by appearance of uniform dark brown & no red colorations (WHO 2010). At 0.5 hr of drying time, the spots were wet even not spread completely on the matrix & bright red in colour. Responses were increased as increase in drying time up to 2.5 hr. The responses observed were same at drying time of 2.5, 3.5 & 4.5 hr. Now blood spots were become dark brown in colour from red colour. After this time, the responses were remaining constant as represented in the figure 1.



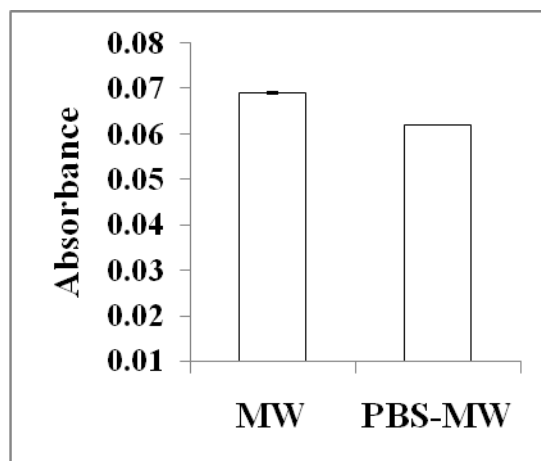
**Figure 1:-** Response of TSH at different time. At 0.5 hr of drying, spot was not possible to punch. At 5.5 hr min of drying spots were too much dried not able to punch. 210 min was the recommended drying time

Similar observations were noticed earlier by Elizabeth et al., 2012 and Hoffman et al., 2013. Some studies mentioned that 6 hr, 12 hrs and overnight drying were necessary for the maximum efficiency of the process (Abdulaha et al., 1999, Hoffman et al., 2013, Lee et al., 2011, Nicolas et al., 2013). After drying analyte contained in a DBS sample must be brought into solution before analysis. A standard hole punch is typically used to cut out discs of whole blood of uniform size, and one is placed into an elution buffer for 5 hrs. Phosphate-buffered saline is a good place to start for an elution buffer as reported in published study (Neto and Schulte J 1998, Abdulaha et al., 1999, Hoffman et al., 2003) but present study reported less responses of TSH. In this study dried blood spots were eluted in distill water, millipore water & phosphate buffere saline (PBS). The responses observed in elution with phosphate buffer saline at pH 7.4 were significantly lower than distill water and millipore water as depicted in figure 2.



**Figure 2:-** Response of TSH in different eluting solvent phosphate buffer saline/millipore water/distill water (PBS/MW/DW) at same Ph 7.4

Responses were increased with increase in pH of the millipore water. Responses of PBS were remains the same, when prepared in distill water as well as millipore water. Figure 3 represented that the response observed in millipore water was remains significantly higher ( $p < 0.0001$ ) as compared to phosphate buffer saline which was prepared in millipore water.

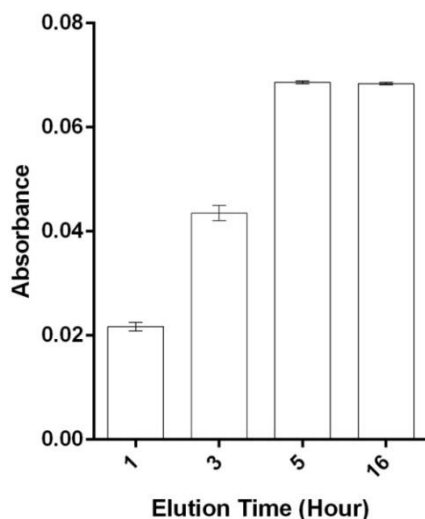


**Figure 3:-** The response of TSH in millipore water was higher as compared to millipore phosphate buffer saline at similar pH 7.4.

The response of TSH in phosphate buffer saline cannot be enhanced whether it was prepared in distill water and millipore water. The response in millipore water remains high as compared to phosphate buffer saline after consideration of all suitable conditions.

Responses were continuously responses decreased when molarities of the PBS increased, maximum response of TSH conc. were observed at the 10 Mm PBS. In present study, millipore water was first time use for the extraction TSH from DBS matrix. Only one study (Lee et al., 2011) is available which used millipore water for the extraction from DBS matrix & determined sensitivity & specificity of HIV Ag/Ab, HBS Ag, anti HBS & anti HCV agents.

The elution efficiency of the solvent increase with increase in time of incubation as depicted in the figure 4.

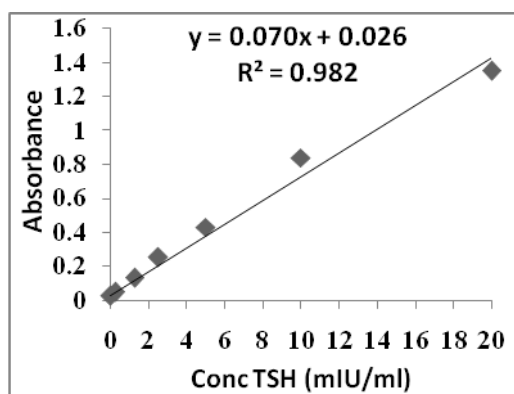


**Figure 4:-** Absorbance of TSH at different incubation time.

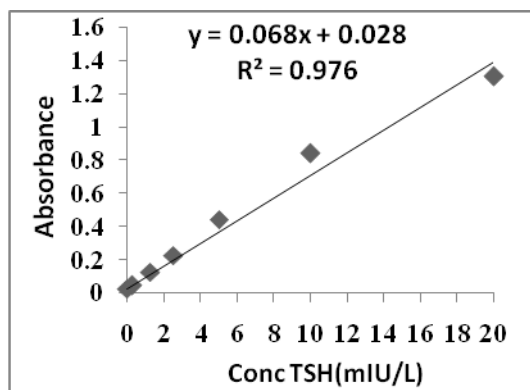
**On the basis of these results incubation time of 5 hr was recommended:-**

Present study also in favors with Takat et al 1998 study short duration (1, 3, 4 hr) of incubation gives lesser responses. This study represented that 5 hr of incubation gives similar responses as that of shown by overnight incubation as depicted in the figure 4. Similar findings were observed by Torresani 1985 study, Neto and Jaqueline 1998, Miller and McDade 2012 study and McDade 2014. Some studies (Larsen et al., 1976) observed that incubation for more than 24 hrs for provides better response. Whatman 903 dbs cards are commonly used for DBS sampling.

In our study whatman 903 dbs card was used to collect the blood specimen and added with different known concentration of TSH (range from 0.25 to 20 mIU/ml). Absorbance measured at 420 nm was plotted as TSH concentration and range of TSH estimation ranging from 0.25-20 mIU/ml . The slope and regression coefficient of the standard curve was found to be 0.070 and 0.982 respectively (figure 6, 7). Although whatman DBS card is quite popular among all dbs substrate for protein estimation. Recovery of TSH from this card was 83-98%. The % mean of interassay variation was 0.40%.The concentration of TSH at 50 % hematocrit were 0.731,1.4,2.15(experimental conc 2.5 mIU/ml ), 6.35,12.71,18.75 (experimental conc 20 mIU/ml) mIU/L on TLC silica matrix after serial dilution (1:3, 1:1.5) of 0. 2.5, & 20 mIU/L of TSH concentration. More importantly, whatman 903 dbs card has disadvantage of cost (70 INR per sample) which is not suitable for use this card for epidemiological study in developing countries.



**Figure 5:-** Calibration Curve of Whatmann 903 dbs card.



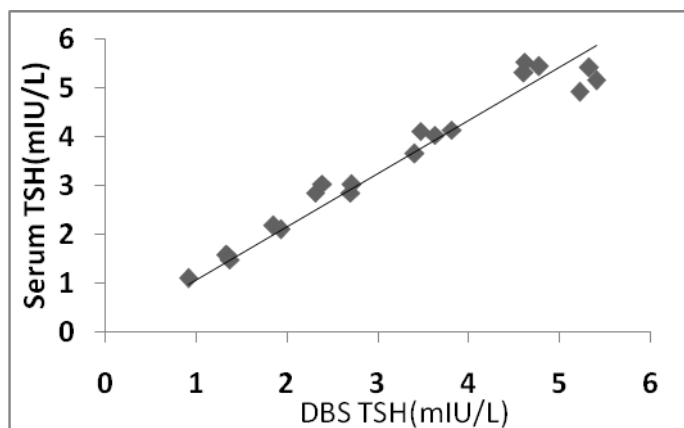
**Figure 6:-** Calibration Curve of TLC matrix

The present method being described here involves the use of TLC silica matrix as DBS matrix. The different conditions for the TLC matrix were optimized and standard curve presented in figure 5. The slope of the curve was 0.068 while coefficient of regression values was 0.976. The % mean of CVs were 7.52%, 5.23%, 2.10%, 2.50%, 2.70% & 1.31% at 0.0.25, 2.5, 5, 10, 20 mIU/L. The % mean of interassay variation was 0.49%. The concentration of TSH at 50 % hematocrit were 0.074, 0.153, 0.210 (experimental conc 0.25 mIU/ml, 0.724, 1.38, 2.11 (experimental conc 2.5 mIU/ml), 6.19, 12.66, 18.99 (experimental conc 20 mIU/ml) mIU/L on TLC silica matrix after serial dilution (1:3, 1:1.5) of 0.25, 2.5, & 20 mIU/L of TSH concentration. These responses followed linearity with slope A= 0.105, B= 1.0866, C= 9.977 & equilibrium constant was 0.497. This matrix is very cost effective in nature which can be easily used for the epidemiological survey works.

The concentration of TSH remains unaffected by hematocrit (30-75%) on Whatmann DBS 903 card & TLC silica matrix. as shown in figure 6 and 7. High values of recoveries shown at high HCT level, similar findings were observed in published studies (Arora et al., 2013, Peng et al., 2012, Denniff and Spooner 2010).

### Comparison of DBS and Serum:-

The correlation coefficient for the two values of same sample (TSH in serum & DBS) was 0.95, (Pearson  $r = 0.97$ ) the slope was 1.013 and the intercept was 0.256. Using  $y = mx + b$  (serum = y; DBS = x) as shown in figure 8. Correlation between TSH DBS and TSH plasma concentrations supports the use of DBS for hormonal studies. The mean level of TSH serum and TSH DBS were  $3.50 \pm 0.342$  &  $3.12 \pm 0.320$  mIU/L and ranges were DBS TSH 0.91-5.32 mIU/L and TSH serum 1.11-5.53 mIU/L. The TSH value calculated by traditional & DBS method shows non significant differences by comparing with unpaired t test ( $p > 0.0001$ ).



**Figure 7:-** Correlation coefficient of serum TSH and DBS values in 20 samples collected from same subjects. Correlation coefficient, slope and intercept are calculated.



## Conclusion:-

This present assay is highly sensitive with a low blood volume requirement & low cost, it is especially attractive for special patient populations such as pregnant population who have conditions that limit the amount of allowable blood obtained for this type of study. This method does not require any buffer preparation for sample incubation. Until now, an alternative DBS matrix has not been available for laboratories to utilize throughout their various stages of processing, collection & storage and cost effective in nature.

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