Research Article

Newborn screening by enzyme immunoassay using dry blood spot for diagnosis of metabolic disorders

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Received: January 02, 2021; **Accepted:** June 20, 2021

ABSTRACT

Background: Newborn screening (NBS) is the test used to diagnosis the metabolic and other disorders within 72 h of birth. The early identification of the disorder helps in early treatment which prevents neonates from life-threatening health problem, mental retardation, and serious lifelong disabilities. **Objectives:** Universal NBS using heel-prick dried blood spot (DBS) samples has become an integral part of public health system in developed countries. There are about 99.9% of newborns that are screened every year in western countries. Whereas, in India the less 1% of newborns are screened due to the ignorance and not knowing the importance of NBS. Very few states in India had started the program in government. Awareness among the professionals and public about the NBS has to be created and extended. **Materials and Methods:** The Newborn DBS samples were received from Government Vellore Medical College Hospital, Government Hospitals at Tirupattur, Gudiyatham and 104 primary health centers. Samples should be collected within 72 h from the newborn. Screening of human thyroid-stimulating hormones, 17-OHP, glucose-6-phosphate dehydrogenase, Galactose, and IRT are done using the Enzyme Immunoassay (EIA) method using their respective conjugate, Conjugate Diluent, Substrate solution TMB Chromogen, washing solution 10 ×, 1 × 50 ml Stopping solution and measured using micro plate photometer. Point of care testing (POCT) requires only 1 drop (10 μL) of blood from the pricked heel of the baby, and it can be performed 5–10 min at bedside. **Results:** NBS is done using the EIA method which reports between 8 and 12 days. Comparing point of care (POCT) with EIA, the results of POCT are compatible and satisfactory and it completes in 5 days. **Conclusion:** The proper implementation of "point of care" testing will help to identify the positive cases early and prevents the baby from permanent disability.

KEY WORDS: Dried Blood Spot; Enzyme Immuno Assay; Heel-prick; Newborn Screening; Point of Care

INTRODUCTION

The word newborn screening ("NBS") helps in diagnosis various disease that occurs within few hours of newborns, which helps in preventing the baby from severe health problem in the future. NBS is considered as a one of the important public health inventiveness of the recent period. NBS is a technique that evolved from simple blood or urine test which mainly used in the identification of inborn error of newborns; it also helps in the identification of 50 other different condition in more detail and complex condition.[1]

Access this article online	
Website: http://www.ijmsph.com	Quick Response code
DOI: 10.5455/ijmsph.2021.04032202119062021	

Inborn Error Metabolism (IEM) is a condition that leads to the severe health problem such as disability and mental retardation, which may cause even death if left undiagnosed and untreated is an important cause of morbidity and mortality in newborns.[2] NBS is done by heel-prick method. The blood sample is collected in the grade 903 filter paper as it was universally recommended dried blood spot (DBS). In

the developed countries, it had become an important part of the health system in the public.[3]

In India – Chennai, Delhi, Kolkata, Mumbai, and Hyderabad are the five centers, where the ICMR initiated a program to screen neonates for inborn metabolic error through a task force. This study took place from 2007 to 2012.[4] The main components of NBS include six vital elements for a successful NBS system. They are Education, Screening, Early follow-up, diagnosis, management, and Evaluation.[5]

History of NBS

NBS task force by American Academy of Pediatrics' in 2000 published a report representing that greater uniformity was needed among programs to assist the professionals, families, and public health agencies.[6] HRSA (Health Resources and Services Administration) American College of Medical Genetics contracted with the Maternal and Child Health Bureau to sketch a process to increase uniformity, which helped in the formation of endorsed uniform panel of conditions.[7]

The quality of test results must be maintained and enhanced in the state health department and laboratories by providing expertise testing, reference materials, consultation, and training assisted through NBS Quality Assurance Program.[8] Center for Disease Control (CDC's) NBS and Molecular Biology Branch have been collaborating with Association of Public Health Laboratories to lecture current trends and advances in molecular testing. This leads to the improvement of data-sharing molecular resources website for molecular assessment and NBS program, here the assessment of molecular capabilities can be received in NBS laboratories.[9]

Importance of NBS

Congenital disorders can be identified within few hours of birth, which can be cured and managed with the help of the NBS. Early identification and treating helps in minimizing or avoids the life-threatening health problem, lifelong disabilities, and mental retardation.[10] The wellness and the health of the newborns might be affected by numerous conditions that present at birth (congenital). These are rare condition; however, some are prevailing in certain families. The disorders such as metabolic disorder, endocrine disorder, abnormal form of hemoglobin and problem in red blood cells carry oxygen protein. There is no exact cure for this condition, but it can be treated, and the child can have a normal life as it grow.[11]

Metabolic Disorders for Newborns

Table 1 is adapted from the SACHDNC Recommended Uniform Screening Panel. Basic seven parameters for

Table 1: Type of disorders

Type of disorder	Disorder
Metabolic: Organic acid	Propionic academia (PROP)
	Methylmalonyl-CoA Mutase deficiency
	Methylmalonic Acidemias
	Isovalericacidemia (IVA) 2-Methylbutryl-CoA Dehydrogenase deficiency
	3-Methylcrotonyl-CoA carboxylase deficiency 3-Methylglutaconyl-CoA Hydratase deficiency
	3-Hydroxy-3-methyglutaryl-CoA Lyase Deficiency
	Holocarboxylase synthase deficiency (MCD)
	ß-Ketothiolase deficiency (ßKT) Isobutyryl-CoA Dehydrogenase deficiency
	Glutaricacidemia type I (GA1) Some Adenosylcobalamin Synthesis Defects Maternal vitamin B12 Deficiency Mitochondrial Acetoacetyl-CoA Thiolase Deficiency Multiple -CoA carboxylase deficiency Malonic Aciduria
Metabolic: Fatty acid oxidation	Carnitine uptake deficiency
	Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
	Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD) Medium/Short Chain Hydroxy Acyl-CoA dehydrogenase deficiency Medium-Chain Ketoacyl-Coa Thiolase Deficiency
	3- Hydroxy Long-chain Acyl-CoA Dehydrogenase Deficiency (LCHAD) Short -Chain Acyl-Coa Dehydrogenase Deficiency Carnitine /Acylcarnitine Translocase Deficiency Carnitine Palmitoyl Transferase Deficiency Type 2 Carnitine Palmitoyl Transferase Ia Deficiency Carnitine Palmitoyl Transferase Ib Deficiency 2,4-Dienoly-Coa Reductase deficiency 1 Glutaric Acidemia type 2
	Trifunctional protein deficiency (TFP)
Metabolic: Amino acid	Argininemia (ARG1 Deficiency) Argininosuccinic Aciduria (ASA Deficiency) 5-Oxoprolinuria 1 Carbamoylphosphate Synthetase Deficiency 1 (CPS 1 Deficiency) Citrullinemia, type I (CIT) Citrullinemia, type 2 Homocystinuria (HCY) Hypermethioninemia Hyperammonemia, Hyperornithinemia, Homocitullinemia syndrome 1 Hyperornithinemia with Gyral atrophy 1
	Maple syrup urine disease (MSUD) N-Acetyl Glutamate Synthetase deficiency (NAGS Deficiency)
	Classic phenylketonuria (PKU)
	Classical/ Hyperphenylalaninemia
	Defects of Biopterin cofactor Biosynthesis
	Defects of Biopterin cofactor regeneration

(*Contd...*)

Table 1: (*Continued*)

Type of disorder	Disorder
	Tyrosinemia (detected by SUAC) Transient Neonatal Tyrosinemia Tyrosinemia, Type I Tyrosinemia, Type 2 Tyrosinemia, Type 3 Ornitine transcarbamolyase deficiency (OCT)
Endocrine	Primary congenital hypothyroidism (CH)
	Congenital adrenal hyperplasia (CAH)
Hemoglobin	S,S disease (Sickle cell anemia) (Hb SS)
	S, ß-thalassemia (HbS/ßTh)
	S,C disease (HbS/C)
Other	Biotinidase deficiency (BIOT)
	Cystic fibrosis (CF)
	Classic galactosemia (GALT)
	Glycogen storage disease type II (Pompe, GSD II)
	Mucopolysaccharidosis type I (MPS I)
	X-linked adrenoleukodystrophy (X-ALD)
	Severe combined immunodeficiences (SCID)
Non-laboratory tests	Critical congenital heart disease (CCHD)
	Hearing loss (HEAR)

screening for metabolic disorders for newbornsare presented in Figure 1. Effects of different types of metabolic disorders of newborns are described in Table 2.[20]

Status of NBS

Status in other countries

In virtually, all the western countries and many countries in Asia, the NBS is carried out. The diagnosis of Phenylketonuria using the filter paper is introduced in 1960 with the help of NBS in USA. The NBS test differs from country to country according to their resources and the incidence condition. There are about 40–50 metabolic disorders in the universal screening which had been made mandatory in US, Europe, and the countries around the world. The screening helps in reducing the morbidity and mortality of the disease though it cost effective its benefits are far exceeding.[12]

At present, all the states require NBS for minimum 29 health condition. The types of test condition in the testing panel are decided by the public health department of each state. Some states also offer to screen for other disorders that are not listed in the testing panel is cost effective which is mostly covered by the insurance.[13] In United States, there are about 4 million newborns every year and almost all of them are screened which result in 99.9% of participation report in each state. There are about 12,500 newborns where diagnosed with any one of the disorders identified by NBS was the latest report of the CDC. The condition can be treated and helps in health development and virtually eliminates death if there is an early diagnosis.[14]

Status in India

Congenital adrenal Hyperplasia (CAH), Congenital Hypothyroidism (CH), hearing loss, and glucose-6-phosphate dehydrogenase (G6PD) deficiency are the conditions for neonatal screening proposed in Indian scenario. Congenital hypothyroidism has a high prevalence and is most significant cause of rational disability. The genital abnormalities morbidity, mortality can be the result if CAH is unobserved at birth. In northern part of countries, the G6PD deficiency has moderately high occurrence and the testing cost is reasonable. The developing countries like India need a huge financial implication for universal screening.[15] In Indian scenario, the screening for hearing and congenital hypothyroidism is must and the other test such as CAH and G6PD deficiency may be included in a phased custom. Sickle cell disease and other hemoglobinopathies screening would be commenced in pockets of great prevalence.[16]

Status in Tamil Nadu

The Institute of Child Health (ICH) and Hospital for Children, Egmore in Tamil Nadu has started screening newborns for two disorders, CH and CAH on pilot basis (Nov 2018, Tamil Nadu Chief Minister had inaugurated this Project). Vellore was selected as the pilot district as the prevalence of CH and CAH was high due to factors such as consanguineous marriages. Nearly 77,000 newborns will be screened for a year in Vellore. Three hospitals – Government Vellore Medical College Hospital and government hospitals at Tirupattur and Gudiyatham – are involved. There were several inborn metabolism disorders that babies could be born with but could be completely cured if diagnosed immediately after the birth. Screening and early intervention would help increase quality of life of children with the condition. NBS for three disorders can be easily introduced (congenital hypothyroidism, CAH, and G-6-PD deficiency).

MATERIALS AND METHODS

Experimental Design

The study was conducted on Newborns screening samples received from Govt. Vellore Medical College Hospital, Govt. Hospitals at Tirupattur, Gudiyatham and 104 primary health centers. The samples were collected by the ward nurses and transferred to The ICH and Hospital for Children (HC), Egmore in Tamil Nadu by speed post.

- Total Number of samples will be processed is 88 for
- Human Thyrotropin (Thyroid Stimulating Hormone, human thyroid – stimulating hormones [hTSH])
- 17- Hydroxyprogesterone (17-OHP)
- G6PD
- Galactose
- Immuno Reactive Trypsinogen (IRT)

Samples should be collected within 72 h from the newborn.

Table 2: Effects of different types of metabolic disorders of newborns

Disorder	Organ or system affected	Signs of metabolic instability and/or clinical considerations	Neurodevelopmental effects (if not treated)
Classic phenylketonuria	CNS	Hyperactivity, seizures	Intellectual developmental disabilities; autism; risk of executive functioning deficits, slow reaction time, and depression
Cystic fibrosis	Lung, pancreas	Failure to thrive, pancreatic insufficiency	—
Classic galactosemia	Liver, CNS	Failure to thrive, poor feeding, lethargy, vomiting, jaundice, infection, liver failure	Intellectual developmental disabilities, cataracts
Biotinidase deficiency	CNS	Episodic hypoglycemia, lethargy	Intellectual developmental disabilities, hearing loss, seizures, hypotonia
Primary congenital hypothyroidism	Thyroid, muscle, gastrointestinal	Jaundice, large fontanelles, pallor, myxedema	Delayed growth and weight gain; hypotonia; developmental delay
Congenital adrenal hyperplasia	Adrenal, gastrointestinal, genitourinary, central nervous system	Poor feeding, vomiting, dehydration, hyponatremia	Intellectual developmental disabilities, learning disabilities, short stature, psychosexual disturbances
G6PD	Blood cells	Jaundice, or yellowing of the skin and whites of the eyes, urine that is dark or yellow-orange, rapid heart rate, anemia, prolonged jaundice	

Adapted from Scriver CR, ed. *The Metabolic and Molecular Bases of Inherited Disease* 8th ed. New York, NY: McGraw-Hill; 2001. G6PD: Glucose-6-phosphate dehydrogenase

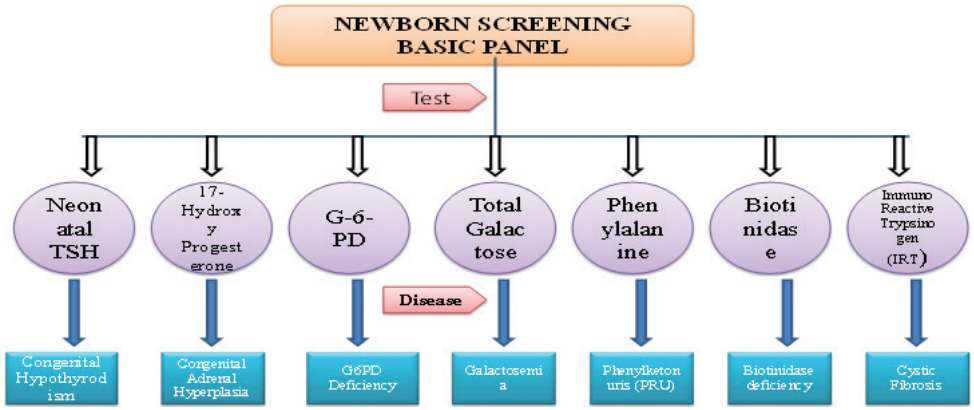

Figure 1: NBS basic panel

Sample Collection

DBS

A blood spot on the filter paper was obtained by one application of the filter paper onto a drop of blood from the pricked heel of the baby 3–5 days after birth. Grade 903 filter paper was recommended for collection of blood spots. Make sure that the filter paper sample was fully covered and soaked through. The blood spot was dried for at least 3 h. Once dried, place each specimen in a separate paper envelope and mail it to the laboratory. Blood spot specimens received in the laboratory should be stored at –2°C to +8°C protected against moisture.

Instrument

N8496 DBS puncher with a diameter of 3 mm to cutoff paper disc of DBS, Thermostar - Microplate incubator/shaker, BioTek - Microplate washer, Microplate disc remover, and BioTek - Microplate photometer.

Methods

Screening of hTSH, 17-OHP, G6PD, Galactose, and IRT are done using the Enzyme Immunoassay (EIA) method using their respective conjugate, conjugate diluent, Substrate solution TMB Chromogen, washing solution 10×, 1×50 ml Stopping solution and measured using microplate photometer.

Point of Care Testing (POCT)

The test requires only 1 drop (10 μL) of blood from the pricked heel of the baby, and it can be performed 5–10 min. Biocard™ Neonatal TSH test is a rapid test for qualitative determination of elevated hTSH in blood specimens as a primary screening of babies for congenital hypothyroidism.

Comparison of EIA and POCT Method

On the way to compare EIA and POCT method, the same blood sample is used to test both EIA and POCT method. The

POCT results were found to be compatible and satisfactory with EIA. POCT is also helps to reduce the turnaround time compared with EIA.

RESULTS

A total number of 88 DBS samples of neonates were selected for the study. The 88 DBS samples of newborn were tested for both TSH and 17OHP by enzyme immuno assay method in which two positive cases were suspected TSH and one positive case was found for 17OHP [Figure 2]. A total number of 88 DBS samples of neonates were selected for G6PD, Galactose and IRT by Immuno assay method and there are no positive cases for this metabolic disorder [Figure 3].

First the DBS samples were collected from the neonates to test for TSH and 17OHP by Immuno assay method. The negative cases are reported once the process is completed whereas for the positive cases, the DBS samples were repeated, and the patients are recalled for the collection of fresh DBS and serum samples. The second DBS samples was tested for TSH and 17 OHP by EIA method for the confirmation of the positive cases and for further confirmation the serum sample is tested by the Chemiluminescence Immuno assay method. When the levels are higher than the biological reference intervals it moves to nest step. Final confirmation of the positive cases of 17OHP and TSH was done by Liquid chromatography and Mass spectrophotometry using the serum sample. Once the positive cases are confirmed, it is reported to the primary consultant and the patient is called again for the treatment and follow-up of the patient should done properly to save the newborn from the permanent disability [Figure 4].

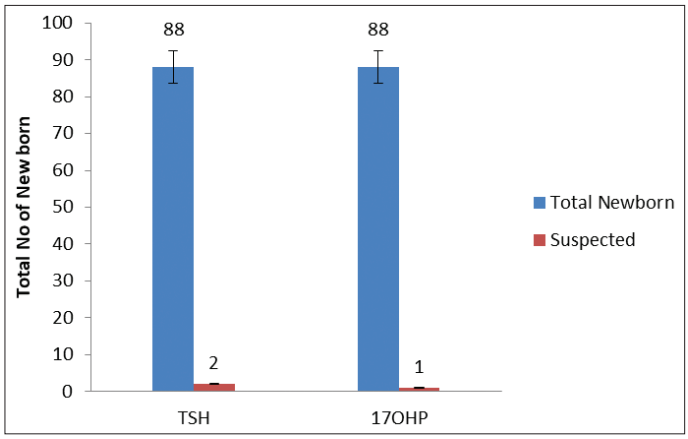

Figure 2: Estimation Of 17-Hydroxy Progesterone (17ohp) and thyroid stimulation hormone (tsh) in dry blood spot by enzyme immuno assay (each bar represent the total no of new born tested and the suspected of metabolic disorders)

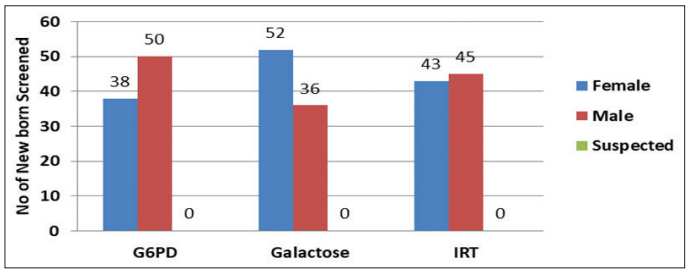

Figure 3: Estimation of glucose-6-phosphate dehydrogenase, galactose, irt in dry blood spot by enzyme immuno assay (each Bar Represent The Total No Of New Born Tested And The Suspected Of Metabolic Disorder)

In general, the DBS samples of newborn were tested for metabolic disorder by EIA method, which takes 12 days to confirm the cases and to start the treatment. Because it needs a day for the sample collection, sample transport, sample registration, and sample processing. In case of positive case, it again needs a day to repeat the DBS sample, call for the baby, repeat sample collection, sample transport, sample processing, and reporting. Then for the confirmatory test by CLIA and LCMS it needs 2 days. To start a treatment for the baby, it needs 12 complete days for the EIA method.

To reduce the days count and to start the treatment early to the affected baby can help to prevent the baby from the permanent disability. The POCT method help in the immediate identification of NBS result as it takes only 5 days to complete confirmatory test for the positive cases [Table 3].

To compare the POCT and EIA method, we used the sample of same baby to test TSH by both POCT and EIA method. POCT results were found to be satisfactory and compatible with EIA. Thus, POCT can be used for NBS [Table 4].

DISCUSSION

Early identification and treatment for the common and genetic disorder through NBS can help to avoid intellectual

Table 3: Analysis of turnaround time for EIA versus POCT

Variables	EIA	POCT
	Day	
Sample collection	1	1
Sample transport	1	
Sample Registration	1	
Sample processing	1	
Reporting	0	
If Positive Repeat	1	
Call the baby for repeat sample	1	
Repeat sample Collection	1	
Sample transport	1	1
Sample processing	1	1
Reporting		
Confirmation by CLIA	1	1
Confirmation by LCMS	1	1
Treatment starts at	12th Day	5th day

EIA: Enzyme immunoassay, POT: Point of care testing

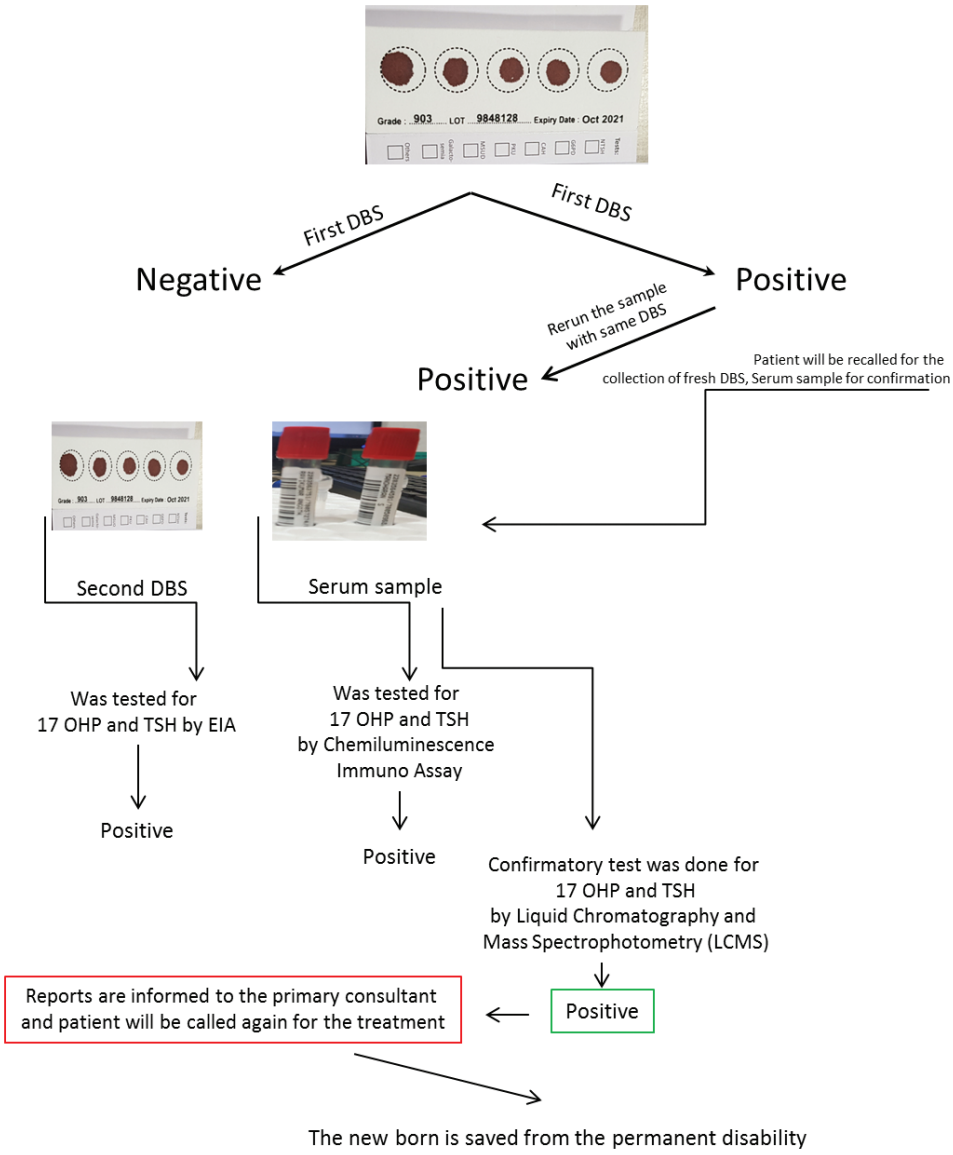

Figure 4: Case study follow-up: Schematic diagram

Table 4: Method comparison of POCT versus EIA TSH

Sample	EIA	POCT
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative
6	Positive	Positive
7	Negative	Negative
8	Negative	Negative
9	Negative	Negative
10	Positive	Positive

POCT Results found satisfactory and compatible with EIA So the POCT can be used or NBS. EIA: Enzyme immunoassay, NBS: Newborn screening, POCT: Point of care testing

physical defects and life-threating illnesses.[17] The word "NBS" helps in doing different tests that occurs in the 48 or 72 h of newborns, which helps in preventing baby from the severe health problem that occurs in the future.[18] IEM (Inborn Errors of Metabolism) of neonates can be diagnosed by NBS for which initial treatment helps to prevent the mental retardation and mortality rate, which is considered as the serious irreversible complication.[19] NBS is done by heel-prick method. The blood sample is collected in the grade 903 filter paper as it was universally recommended DBS. In the developed countries, it had become an important part of the health system in the public.[3]

In United States, there are about 4 million newborns every year and almost all of them are screened which result in 99.9% of participation report in each state. However, in India, the screening was done to <1% which needs to be improved.[14] In Tamil Nadu, the NBS was done in very limited amount and only in specific labs. Hence, there is lack of awareness about the importance of the NBS. The blood sample is collected from the pricked heel of the baby 3–5 days after birth. The

specimens are then sent to a state designated NBS laboratory for analysis. When a test result is out of normal range, laboratory or follow-up personnel contact the birthing facility and the newborn's physician to ensure the child receives the appropriate diagnostic work-up and treatment.

Due to limited labs for NBS testing the test report for the cases takes longer time than usual, which makes it difficult to follow-up the patient and give treatment. To avoid this conflict, the implementation of "point-of-care" testing for the metabolic disorders helps in immediate identification for screening; if the sample looks a susceptible for any disease then the baby can be analyzed for further assays. If the NBS is positive, then further confirmation can be done using the Immuno assay method. This will help to follow-up the positive babies rapidly and easily and provide the treatment as soon as possible without any delay. Act fast will increase the percentage of the recovery and avoid further onset of complications which may occur in adulthood.

Strengths and Limitations of Study

- This study helps in comparing the EIA and POCT method of testing, which aids in reducing the turnaround time leads to identification of the disorder and prevent newborn by early treatment
- Serum samples were used to confirm the positive cases of the newborn by LCMS method
- DBS samples were received from a particular region that is used for the testing.

CONCLUSION

NBS helps in identification of the metabolic disorder in the early stage and baby can be prevented from the permanent disability. Some of them are CAH, congenital hypothyroidism, etc. This can be universally tested using Dry Blood Spot which is approved by the CDC. In this study, two positive cases for TSH and one case for 17OHP has been confirmed by the Immuno assay method (CLIA, LCMS) and given treatment to the patients by the proper follow-up of the neonates. There is a delay in turnaround time in EIA, as one of the analysis we did the TAT analysis which reveals that POCT implementation helps the baby to come for treatment on 5th day, whereas by EIA it takes 12 days. The proper implementation of "point of care" testing will help to identify the positive cases early and prevent the baby from future deficiency which will occur due to the delay in treatment.

REFERENCES

1. Driscoll CJ, McPherson B. Newborn Screening Systems: The Complete Perspective. San Diego, CA: Plural Publishing, Inc.; 2010. p. 133-56.
2. Kaur G, Srivastav J, Jain S, Chawla D, Chavan BS, Atwal R, et al. Preliminary report on neonatal screening for congenital hypothyroidism, congenital adrenal hyperplasia and glucose-6-phosphate dehydrogenase deficiency: A Chandigarh experience. Indian J Pediatr 2010;77:969-73.
3. Fölling AU. Excretion of phenylpyruvic acid in the urinary metabolism abnormality in connection with imbecility. Z Physiol Chem 1934;277:169-76.
4. ICMR Multicentric Study: Newborn Screening for Congenital Hypothyroidism and Congenital Adrenal Hyperplasia and High Risk Screening of Infants. National Task Force of Inborn Metabolic Disorders, Draft Report; 2014.
5. Serving the family from birth to the medical home. Newborn screening: A blueprint for the future-a call for a national agenda on state newborn screening programs. Pediatrics 2000;106:383-422.
6. Lloyd-Puryear MA, Tonniges T, van Dyck PC, Mann MY, Brin A, Johnson K, et al. American academy of pediatrics newborn screening task force recommendations: How far have we come? Pediatrics 2006;117:S194-211.
7. Therrell BL, Hannon WH. National evaluation of US newborn screening system components. Ment Retard Dev Disabil Res Rev 2006;12:236-45.
8. Centers for Disease Control and Prevention (US). Newborn Screening Quality Assurance Program. United States: Centers for Disease Control and Prevention; 2013.
9. Association of Public Health Laboratories. Newborn Screening Molecular Resources. United States: Association of Public Health Laboratories; 2013.
10. Newborn Screening Tests, Kids Health; 2017.
11. Newborn Screening Laboratory Bulletin, Centers for Disease Control and Prevention; 2014.
12. Wilson JM, Jungner G. Principles and Practice of Screening for Disease. World Health Organization Public Health Papers No. 34; 1968.
13. President's Council on Bioethics. The Changing Moral Focus of Newborn Screening: An Ethical Analysis by the President's Council on Bioethics. Washington, DC: President's Council on Bioethics; 2017.
14. Martin JA, Hamilton BE, Osterman MJ, Osterman MH, Driscoll AK. National vital statistics reports. In: Births: Final data for 2015. Vol. 66. United States: United States Department of Health and Human Services; 2017. p. 1-67.
15. Kaur M. Newborn screening: The critical importance. Indian Pediatr 2014;51:698-9.
16. Mukhopadhyay K, Balachandran B. Universal newborn screening-is it going to be a reality in India? Indian Pediatr 2014;51:697-8.
17. Boyle CA, Bocchini JA Jr., Kelly J. Reflections on 50 years of newborn screening. Pediatrics 2014;133:961-3.
18. Bradford LT. Newborn screening systems. In: Newborn Dried Bloodspot Screening. The Complete Perspective. San Diego, CA: Plural Publishing, Inc.; 2010. p. 133-56.
19. Ryff CD, Singer B. The contours of positive human health. Psychol Inq 1998;9:1-28.
20. Scriver CR, editor. The Metabolic and Molecular Bases of Inherited Disease. 8th ed. New York: McGraw-Hill; 2001.

How to cite this article: Fahima Sheerin SMH, Divya S, Chindhiha S, Vincy S, Chandrasekar M, Selvakumar K. Newborn screening by enzyme immunoassay using dry blood spot for diagnosis of metabolic disorders. Int J Med Sci Public Health 2021;10(1):107-113.

Source of Support: Nil, **Conflicts of Interest:** None declared.