TECHNICAL SERIES

Newborn Screening

Importance & Challenges

TULIP DIAGNOSTICS (P) LTD.

...Setting trends

Other Technical Series published by Tulip Diagnostics (P) Ltd.

1. Antimicrobial Susceptibility Testing- Practical Issues.
2. Malaria RDT's Performance Evaluation and Quality Assurance-Perspectives.
3. Quantia Clinical Applications.
4. Microkrom Microscopy Stains-Basic Guidelines for Quality Staining with Romanowsky Stains.
5. Cardiac Troponin I (cInI)-New Generation Cardiac Marker of Choice.
6. Glycated Hemoglobin (GHb)-The marker for retrospective glycemic control.
7. Hepatitis C Virus-Perspective.
8. ELISA-Troubleshooting Aspects.
9. Turbidimetry.
10. Human Immunodeficiency Virus-Perspectives.
11. CK and its isoenzymes-The Time Tested Biomarker for Diagnosis and Monitoring of MI.
12. Malaria and its diagnosis-Rapid test for Malaria Detection.
13. Lupus Anticoagulants-Basic Concepts and Laboratory Diagnosis.
14. Syphilis Diagnosis.
15. Monitoring oral anticoagulant therapy-Concepts & Practice.
16. Quality Assurance for routine Hemostasis Laboratory.
17. Mycobacterium Tuberculosis-AFB Staining, Culture.
18. Anti-Human Globulin Reagent-Basic Concept & Practice.
19. HIV Testing-Evaluation & Practical Issues.
20. Inherited Metabolic Disorders and Newborn Screening- An Insight

Foreword

Since its Inception in 1988, Tulip Diagnostics (P) Limited, a leading Indian manufacturer of in-vitro diagnostic reagents, kits and instruments has been expanding and growing in its business at a very fast pace. With nine ISO 13485 certified manufacturing plants pan India and exports to over 88 countries worldwide, the company and its divisions are continuous evolving into newer areas of diagnostics including Newborn Screening assays.

Tulip Diagnostics has always believed that knowledge upgradation is the fundamental basis for better diagnosis and patient care. Publishing of Technical series is one such initiative to make available to the laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day to day practice.

Newborn screening (NBS), in India as well as in many emerging countries, is at a nascent stage. There is a need for increasing awareness about the utility of Newborn screening in these population. There is also limited public awareness in India and in these countries about the need to test apparently healthy newborns for life-endangering congenital disorders. Such screening will help in administering timely treatment to prevent medical and psychosocial complications and improve disease outcomes and decrease the associated morbidity.

In this tech series we provide a brief insight into Newborn screening - its current worldwide status, the Indian scenario, National Neonatology Forum's advocacy on the minimum set of NBS disorders to be screened nationally, the challenges faced by the developing countries over implementation of Newborn screening and what can be done to overcome these challenges.

It must be noted that the information provided in this edition is intended as a guide. We hope the contents of this tech series is informative and useful.

Happy Reading!

CONTENTS

NEWBORN SCREENING

Definition:

Newborn screening (NBS), an essential Public Health Program and now an integral part of Neonatal care, is a process of testing all babies soon after their birth for certain disorders (Inherited metabolic disorders) or conditions which are not apparent at birth, but can be life threatening or affect the normal development of the child. In the NBS program all the babies are tested, irrespective of whether they have the disorder or not.

"**Catch them Early**" aims to identify early before significant morbidities sets in. Screening is a process of filtration. It separates the new born infants into two groups, one who may have the disorder or condition, the other who do not have it. If the screening test result is positive for a given disorder or disease, it should be followed by a confirmatory diagnostic test. If the diagnostic test confirms the case, then the specific treatment has to be implemented.

INHERITED METABOLIC DISORDERS (IMD)

Inherited metabolic disorders (IMD) constitute more than 5000 different single gene disorders/ conditions of which many, are all individually rare, but reach a cumulative incidence of approximately 1:1600-2000 newborns. The medical term coined for these disorders is Inborn Errors of Metabolism (IEM). [1]

IEM conditions could be life threatening and /or cause physical and/or intellectual or global developmental disability. If these patients are not diagnosed and treated early in life, this may lead to irreversible brain damage. Many body systems are affected, and the predominant damage could be to the central nervous system (CNS). With the result the babies may develop permanent mental retardation, growth retardation, intractable seizures, cerebral palsy etc. Most of these conditions may present in the first few days of postnatal life but may also become apparent with age. The younger the child more diffused is the clinical presentation. Patients are mostly critically ill during the metabolic crisis and death is not unusual. If they survive, the crisis often leaves the patient with sequelae. [5,6,7]

Prognosis is highly dependent on early diagnosis and introduction of specific treatment regimens and management.

With advancing techniques of early detection and a special dietary management, severe brain damage can be prevented/avoided, and the outcome of the patients changes dramatically.

IEMs are mostly inherited as autosomal recessive traits and are due to an enzyme or transport protein defect. This defect can lead to deficiency and/or an accumulation of specific metabolites. Inability to eliminate the precursors or the deficiency of essential products is directly or indirectly responsible for disease manifestations and clinical course. Some of the genetic metabolic disorders are also related to pathways of amino acid metabolism, fatty acid oxidation metabolism, organic acid metabolism, glycogen storage, and lysosomal storage.

To avoid irreversible clinical defects of IEMs especially in the first postnatal days of life, a newborn screening technique was developed by Dr. Robert Guthrie in 1960 starting with Phenylketonuria as the first disease. Phenylketonuria became a treatable disease and patients with this amino acid disorder could live normal healthy life. [7,9]

Congenital hypothyroidism (CH) and Congenital Adrenal Hyperplasia (CAH) followed as the next screening disorders and many others have since become a part of the screening tests.

***IEM:** A Medical term coined as Inborn Error of Metabolism for inherited Metabolic disorder.

IMPORTANCE OF SCREENING

Newborn Screening is important as it helps to identify babies with serious medical condition, before appearance of the symptoms and thus aids in early medical intervention and corrective treatment. Most babies with these conditions, if identified at birth and treated early, are able to grow up healthy with normal development. Such early detection allows immediate treatment, which reduces or even eliminates the neurological effects of the condition. Many of the conditions detectable in New-born screening, if left untreated, have serious symptoms and effects, such as lifelong nervous system damage; intellectual, developmental, and physical disabilities; and even death. This extracts a high medical and social cost on the patient, family and society at large.

As per recent data 140 million children are born every year around the world. Of these 5 million die in the first month of life in the developing countries. 4 million children have congenital problems, 25-30% of these have IEMs. Newborn screening will be useful to prevent disability and death by early intervention, follow-up and genetic counselling. Though screening is a cost-intensive exercise, the benefits far exceed the costs as it helps in reducing the morbidity of the disease. [12,13,14]

Blood Collection for NBS Screening:

The preferred time of blood collection is post 48-72 hours of Newborn baby's age. If the initial specimen is collected before 24 hours of age, a second specimen must be collected within 2 weeks of age. [33]

Methods used for NBS Sampling & Testing:

A Heel stick / Heel prick method is used to collect small sample of blood. The health professional will put drops of blood onto the specialized filter paper card to create several "Dried blood spots." The Newborn screening card is then sent to the laboratory for analysis. Depending on the screening parameters and tests various techniques like ELISA/FEIA/ TMS (MS/MS) are used. [22]

Seven Major Components of Successful Newborn Screening Implementation:

Education: Healthcare Professionals, parents and policy makers.

Screening: Collection activities, Specimen delivery, Laboratory testing and Result reporting.

Diagnosis: Interpretation of results & reporting.

Conveying: To doctor & parents, Counselling.

Early Follow-up: Repeat test if positive & confirm.

Management: Medical management, long term follow-up, Specimen management.

Evaluation: Program quality assurance, outcome & cost effectiveness.

NBS - GLOBAL STATUS

After Prof. Robert Guthrie conceived the concept in 1960 in USA and the first metabolic disorder that was tested was **Phenylketonuria**, the development of dried blood spot test facilitated mass screening of babies for this disease and PKU testing became mandatory in all the states of USA. Later, more tests were added to this screening program. Since 2006, in US about 29 conditions have been identified as core panel for universal NBS as a public health program and is being continuously enlarged. [10]

While nationally managed Newborn screening (NBS) program do not exist in either USA or Canada for that matter, there are state, provincial and territorial NBS programs which are well established similar to national programs in other countries. The success of blood spot newborn screening in the USA has led to early screening efforts in the parts of Asia Pacific region from the mid-1960s onwards. Nearly 4 million newborns are screened by filter paper blood spots tests, and almost 12,500 infants with various congenital disorders are diagnosed and treated each year.

Although individual IEM is rare, the collective incidence of IEMs is high and varies dramatically in different countries and regions (Campeau et al., 2008). For example, the incidence of IEM was reported to be 1/666 in Saudi Arabia (Moammar et al., 2010), 1/784 in United Kingdom (Sanderson et al., 2006), 1/1400 to 1/5000 in the US, 1/5000 in Thailand [4] , 1/2500 in Canada (Applegarth et al., 2000), 1/2900 in Germany (Lindner et al., 2011), 1/1944 in Egypt (Hassan et al., 2016), 1/2916 in Malaysia (Yunus et al., 2016), 1/2800 in South Korea (Yoon et al., 2005), and 1/3165 in Singapore (Lim et al., 2014) . The incidence of IEMs is much lower in Japan, approximately 1/9000 (Yamaguchi, 2008; Yamaguchi et al., 2013). [3]

In Japan NBS was introduced in 1977 for five IEMs, PKU, MSUD, Histidinemia, Homocystinuria and galactosemia. With the addition of Congenital Hypothyrodism (CH) as screening test, almost 100% of the newborns undergo NBS for CH. Asia-Pacific countries like Australia, New Zealand and Taiwan included expanded metabolic screening with MS/MS in their screening panels. China, Korea, Thailand, Philippines and Singapore have well planned screening programs supported by the Department of Health. Out-of-hospital births remain a challenge in Bangladesh (80%), India (61%), Philippines (62%), Pakistan (80%), Laos (85.7%), Iran (34.4%), Palestine (38.8%) and Yemen (50%). However, noteworthy is the implementation of newborn screening program in China with a successful coverage of over 85% of all Chinese newborns. Philippines follows China with 65% coverage and implementation of expanded screening with MS/MS. In Latin American countries like Chile, Brazil, Mexico, Argentina, Colombia and Venezuela NBS screening

extend beyond those established by national and regional programs. NBS programs in Cuba, Costa Rica, Uruguay and Chile have been functioning for the last 20-30 years. The screening panels in NBS programs in Brazil, Mexico and Argentina have been increased significantly with expanding coverage.The Middle East with successful national NBS programs are Bahrain, Egypt, Iran, Israel, Kuwait, Oman, Qatar, State of Palestine, Saudi Arabia and UAE. Pilot screening programs exist in Libya, Morocco, Yemen and Algeria, however not much is known about the NBS activities in Sudan and Somalia.

Newborn screening by MS/MS technology has been successfully implemented in most of the European countries (Austria, Belgium, Denmark, Germany, the Netherlands, Poland, Portugal, Spain, Switzerland, and the United Kingdom.

Country wise list of common disorders which are screened [1]:

Countries	Main IEMs disorders screened for full population
U.S	(Phenylketonuria, Congenital Hypothyroidism, Galatosemia, Hemoglobin, Congenital Adrenal Hyperplasia, Biotinidase deficiency, Cystic Fibrosis) – implemented in all 53 States. 5 core fatty acid disorders (51 States), 6 core aminoacid disorders (49 States) and 9 core organic acid disorders (49 States), Severe combined immunodeficiency – SCID (31 States).
Canada	(Phenylketonuria, Congenital Hypothyroidism, MCAD-Medium- chainacyl-CoAdehydrogenase) – in all 15 territories. Cystic fibrosis (13 territories) Sickle cell anemia (8 territories), Galactosemia (8 territories), Congenital Adrenal Hyperplasia (5 territories), Biotinidase deficiency (7 territories), 6 core amino acid disorders (9 territories).
Latin America	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Congenital Adrenal Hyperplasia, Biotinidase deficiency, Cystic fibrosis. (Amino acid disorders, Fatty acid oxidation disorders, organic acid disorders, hemoglobinopathies only in Costa Rica).

Countries	Main IEMs disorders screened for full population
European countries	Congenital Hypothyroidism, Phenylketonuria (more than 40 States). Congenital Adrenal Hyperplasia (19 states), Galactosemia (10 States), Sickle cell disease, Biotinidase deficiency (12 States), Cystic fibrosis (16 States) (5 core amino acid disorders (22 States), 5 core fatty acid oxidation disorders (17 States), 9 core organic acid disorders (17 States).
Middle east and North Africa	Congenital Hypothyroidism.
Bangladesh	Congenital Hypothyroidism.
Australia	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Homocystinuria, Cystic fibrosis, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
New Zealand	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia Homocystinuria, Cystic fibrosis, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
Thailand	Congenital Hypothyroidism, Phenylketonuria.
Malaysia	Congenital Hypothyroidism, G6PD deficiency.
China	Congenital Hypothyroidism, Phenylketonuria.
Hong Kong	Congenital Hypothyroidism, G6PD deficiency.
Japan	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia Homocystinuria, Histidinemia Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.

Countries	Main IEMs disorders screened for full population
Taiwan	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia, Homocystinuria, G6PD deficiency, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
Singapore	Congenital Hypothyroidism, Phenylketonuria, Maple Syrup Urine disease, Homocystinuria, G6PD deficiency, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
Philippines	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia, G6PD deficiency.
South Korea	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia, Homocystinuria, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
India	Congenital Hypothyroidism, Phenylketonuria, Galactosemia, Maple Syrup Urine disease, Congenital Adrenal Hyperplasia, Homocystinuria, G6PD deficiency, Sickle cell anemia, Other amino acid disorders, Fatty acid oxidation disorders, organic acid disorders.
Pakistan	Congenital Hypothyroidism.
Sri Lanka	Congenital Hypothyroidism.

NBS - INDIAN SCENARIO

India has a relatively high birth rate of approximately 24 million babies born every year. India also has a high prevalence of consanguineous (marriages within the family, maternal or paternal side, blood relations) marriage across the country which gives an indication to the possibility of high occurrence of genetic disorders. Annually an estimated 4,95,000 infants are born with congenital malformations, 3,90,000 with G6PD deficiency, 21,400 with Downs syndrome, 9,000 with thalassemia, 9,760 with amino acid disorders and 5,200 with sickle cell anaemia. [2] In India, the prevalence of IEMS is 1:2497. [4]

A screen of 1,12,269 neonates for amino acid disorders in the Karnataka showed four disorders to be the most prevalent: maple syrup urine disease and phenylketonuria, tyrosinemia (with a combined frequency of 1:2495). [2] In a hospital-based study in India, biochemical screening of 4,400 cases of mental retardation revealed 256 cases (5.7%) of amino acid abnormalities. [2] A large number of inborn errors of metabolism (IEM) in children remain undetected in India due to lack of investigative facilities and economic restraints.

The ICMR through a Task Force on Inborn Metabolic Disorders, initiated a program to screen neonates in 5 centres in India- Delhi, Mumbai, Chennai, Hyderabad and Kolkata from the year 2007 to 2012. The overall number of new-borns enrolled for the screening were 1,03,849 for CH and 1,03,712 for CAH. The incidence of CH was 1:130 new-borns, varying 1:727 in Chennai to 1:1528 in Mumbai. The incidence of CAH overall was 1:5762 new-borns, varying from 1: 2,036 in Chennai to 1: 9,983 in Mumbai. [3,4,5,6]

In the ICMR multicentric study 708 high risk infants were also tested at the five centres by TMS (Tandem Mass Spectroscopy) and 16 MSUD cases were detected.

At Sir Ganga Ram Hospital, Delhi, a NBS had been carried out for 3 disorders since 2006, congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH) and G6PD deficiency. Among 16,832 newborns screened up to August 2014, the incidence of CH was 1: 1870, of CAH 1:4208, and for G6PD deficiency 1:263 in males and 1:402 in females. [5]

A study by Dr Radha Rama Devi in the capital city of Andhra Pradesh reveals a high prevalence of treatable IEMs in the state. In this study congenital hypothyroidism was found to be the most common disorder about 1 in 1700 new-borns, followed by congenital adrenal hypothyroidism 1 in 2575 and amino acid disorders to the extent of 1 in every 1000 newborns. [6]

A study of neonatal CH by Kapil U. Jain and his colleagues in the sub-Himalayan area of

Himachal Pradesh revealed that among the 613 umbilical cord blood samples of neonates collected on filter papers and analysed for TSH, prevalence of Neonatal Hypothyroidism was found to 4.4%. [23]

It is observed that the incidence of congenital hypothyroidism in India is higher than the Western countries due to high prevalence of iodine deficiency in many areas (Kochupillai and Pandav, 1986). Subsequent to the implementation of iodization program by Government of India, the incidence of CH was observed to have dropped down in the state of Uttar Pradesh from 100/1000 to 18/1000. [7]

In a multicentric study carried out by the Indian Council of Medical Research (ICMR) in five centres to determine the genetic etiology of mental retardation, 65 out of the 1314 cases had metabolic disorders. Of these, 7 were found to have amino acid disorders (ICMR, 1991). In one study Kaur et. al screened 4451 cases for IEMs in Delhi and detected 4 cases of PKU. In another study conducted from 1986-1992, Kaur et.al screened 2560 referred cases of IEMs from different parts of India (majority from northern India) and 12 cases of MSUD and 2 cases of PKUs were detected. [29]

Dave and Ranjan presented gas chromatography-mass spectrometry (GC-MS) studies on urine on 254 high risk infants in Mumbai and among other disorders, 3 cases of galactosemia were detected. [28]

The state of Goa has the distinction of the starting the first national state new-born screening program “heel to heal” in June 2008. Over the 5- year period ending 14th June 2013, 48,118 infant samples were processed from 3 Government hospitals and 13 Community / Primary health centres. There were 6 confirmed/85 presumptive positive cases of fatty acid oxidation disorders, 5/9 cases of amino acidurias, 4/14 cases of organic acidurias, 17/11 cases of CH, 15/32 cases of G6PD deficiency, 2/10 of CAH. [5]

Cystic fibrosis cases have been reported rare in Indian population with prevalence 1/43,321 to 1/100,323. However a study conducted in Hyderabad showed maximum prevalence of cystic fibrosis, galactosemia and biotinidase deficiency as mentioned in 'Prevalence of inborn errors of metabolism in neonates' by Preeti Sharma et.al. [12]

The same article reports a cross-sectional population-based study conducted at Preventine Life Care Laboratories, Navi Mumbai, Maharashtra, India. The study was conducted for 3 years from October 2012 to November 2015. Mass screening of new-born samples was done via TMS/GCMS/ Enzyme assay/HPLC/ELISA technique. In that study 70,590 samples were analysed; 2053 cases of IEMs were detected (2.9%). Of these

positive cases, 13% (279 of 2053 positive cases) cases belonged to eastern zone, 24% (493 of 2053 positive cases) were from northern zone, 38% (793 of 2053 positive cases) were from southern zone and 23% (488 of 2053 positive cases) were from western zone. Among these, the highest prevalent disorder was found to be G6PD deficiency, with 1.3% (923 positive of 70,590) cases reported followed by haemoglobinopathies, 0.5% (360 positive of 70,590) and congenital hyperplasia with 0.34% (239 positive of 70,590) cases of the total new-borns, screened.

MSUD cases detected were 26 out of 2053 IEMs positives. Cystic fibrosis cases were 71 (East- 7, North 4, South 49 and West 11). Biotinidase deficiency cases were 41 (East- 12, North 9, South 14 and West 6) and Phenylketouria 7 cases. [12]

After Newborn screening was initiated, as a pilot screening project in Bangalore, Karnataka in the year 1980,[6] India has seen a lot of change in terms of evolution and implementation of New-born screening as pilot projects for few disorders. However, there is paucity of published studies in the normal New-born population screening from India. As per the available data, Congenital Hypothyroidism (CH), Congenital Adrenal Hyperplasia (CAH) and Glucose-6- Phosphate Dehydrogenase Deficiency (G6PD) are the three conditions identified to be highly prevalent in the Indian subcontinent and therefore neonatal screening for these 3 disorders has been proposed to be must in Indian scenario [12] G6PD deficiency has a relatively high incidence in northern parts of the country [31] whereas the incidence of CAH is higher in the southern states. [32] Sickle cell disease, common in the tribal belts of central and western regions of India, poses a considerable health burden. This data does not undermine the existence of the other IEMs such as Maple Syrup Urine Disease (MSUD), Biotinidase Deficiency, Galactosemia (TGAL), Phenylketonuria (PKU) which is also there but not much data is available online except for few high-risk studies and case studies on 1 or more samples.

Even if we consider a meagre 2% of live births in India to develop metabolic genetic disorders, then 500,000 affected children are born every year.

India is going through a progressive transitional phase of control over infant mortality and morbidity due to infections, and emergence of genetic conditions. India has registered a significant decline in Infant Mortality Rate (IMR), to a level of 47/1000 live births. It has been seen that as infant mortality goes down proportionally the death from birth defects would rise. Many states have achieved an IMR less than 30/1000 live births. [8] WHO has recommended that genetic services should be introduced in countries with an infant mortality rate (IMR) less than 50. Introducing NBS is akin to introducing genetic services in the country. Though many states within India are implementing pilot programs for

Newborn screening through the generous support of state governments and other organizations. In absence of a national policy regarding neonatal screening in India, it is important to introduce pilot studies in different parts of the country to assess the feasibility of a national screening program. The timely detection/screening of metabolic disorders entails a one-time cost and facilitates effective treatment and management, the benefits of which lead to healthy and disability free lives.

CORE AND EXPANDED NEW-BORN SCREENING

The term '**Core**' panel of disorders indicates the minimum set of disorders (e.g., CH-TSH, CAH-17OHP, G6PD) for which the screening should be advocated at a national level. Since all countries chose the set to be initiated in their country based on the epidemiologic prevalence and resources, the panel of disorders chosen around the world is not uniform. The first criterion for inclusion of a disorder for screening is that the disease should be of magnitude to qualify to be a significant public health problem for e.g Congenital Hypothyroidism.

With the availability of multi-analyte testing by tandem mass spectroscopy (MS/MS) the term Expanded Newborn screening emerged. The modality of the sample collection is the same as for core set i.e, dried blood spot on a filter paper, as for the core set of disorders. The simultaneous screening of multiple analytes from the same drop of blood by MS/MS paved the way for Expanded Newborn screening. The introduction of MS/MS has led to the expansion of disorders that could be screened. [10,22]

Commonly Screened Disorders: [22]

1. Congenital hypothyroidism (CH)- TSH
2. Congenital adrenal hyperplasia (CAH)- 17OHP
3. Phenylketonuria (PKU)
4. Glucose-6-phosphate dehydrogenase (G6PD) deficiency- G6PD
5. Galactosemia (GAL)
6. Maple syrup urine disease (MSUD)
7. Biotinidase deficiency
8. IRT and so on…

NATIONAL NEONATOLOGY FORUM (NNF)

National Neonatology Forum (NNF) which came into existence in India, in 1980 through

the initiative of a handful of leading paediatricians working in the field of neonatology is a strong and large body of over 8000 neonatologists across India & abroad. NNF has been actively engaged in advocacy, policy making, research and ensuring quality healthcare to newborn for last 4 decades.

As per the NNF recommendations, the disorders to be screened in India have been classified into three groups depending on availability of resources. [33]

Group A (All newborns): Congenital Hypothyroidism (CH), Congenital Adrenal Hyperplasia (CAH), G6PD Deficiency are the disorders that can be strongly recommended in the routine newborn metabolic screening in our country due to following reasons: 1. High incidence, easily missed at birth, definitive treatment available, definitive test available to diagnose the conditions. 2. Cost of diagnosis would be quite economical. 3. If missed early in the neonatal period, the child could end up having irreversible damage (CH, CAH). 4. In case of G6PD deficiency the drugs provoking the haemolysis could be avoided. 5. Treatment of these disorders is affordable in most settings in the present scenario.

Group B (Screening in the High-Risk Population): The following disorders can be screened in the high-risk population (Previous children with unexplained mental retardation, seizure disorder, previous unexplained sibling deaths with features suggestive of IEM, critically ill neonates, new-borns/children with symptoms/signs/ investigations suggestive of IEM and consanguinity)

I Phenylketonuria (PKU) **I** Homocystinuria **I** Alkaptonuria **I** Galactosemia (TGAL) **I** Sickle-cell anaemia and other hemoglobinopathies **I** Cystic fibrosis (CF)** **I** Biotinidase deficiency (BIOT) **I** Maple syrup urine disease (MSUD) **I** Medium chain acyl coenzyme Adehydrogenase deficiency (MCAD) **I** Tyrosinemia **I** Fatty acid oxidation defects.

** The screening for CF could be restricted to the high-risk neonate with Meconium ileus in the neonatal period or previous sibling with cystic fibrosis. There are many metabolic disorders that can be diagnosed in neonatal period, however currently the treatment options are not easily available in our country. Many of these disorders require special diet and long-term monitoring for preventing complications. However, the detection of these disorders early in life is useful in genetic counselling of the affected family, which in turn can help prevent the recurrence of similar births.

Group C (Screening in Resource Rich Settings): 'Expanded Newborn Screening' for 30-40 inherited IEM's done by TMS can be offered to the 'well to do' especially in urban settings where facilities for sending samples to the TMS laboratory are available.

NBS Core group of parameters are

- Congenital Hypothyroidism
- Congenital Adrenal Hyperplasia
- Glucose-6-phosphate dehydrogenase deficiency

Reasons for Inclusion:

(a) Minimum set of NBS disorders for which the screening should be advocated at a national level.

(b) Relatively higher prevalence rate in India & rest of the World

(c) Easily missed at the time of Birth

(d) Irreversible clinical consequence if missed e.g., mental retardation, death

(e) Affordable treatment options are available

CONGENITAL HYPOTHYROIDISM (CH)

Congenital hypothyroidism (CH) is defined as thyroid hormone deficiency present at birth. Congenital hypothyroidism is classified into **permanent and transient CH**.

Permanent CH refers to a persistent deficiency of thyroid hormone that requires life-long treatment.

Transient CH refers to a temporary deficiency of thyroid hormone, discovered at birth, but then recovering to normal thyroid hormone production. Recovery to euthyroidism typically occurs in the first few months or years of life.

Thyroid hormone deficiency at birth is most commonly caused by a problem with thyroid gland development (dysgenesis) or a disorder of thyroid hormone biosynthesis (dys-hormonogenesis). These disorders result in primary hypothyroidism. Secondary or central hypothyroidism at birth results from a deficiency of thyroid stimulating hormone (TSH). [24]

Congenital hypothyroidism (CH) is defined as partial or complete loss of function of the thyroid gland that affects infants from birth (congenital). Babies with congenital hypothyroidism do not produce enough thyroid hormone to grow and develop normally.

Permanent neurodevelopmental deficits are known to occur when CH is not recognized and adequately treated by 2 to 3 months of postnatal age. Around the world, the most common cause of congenital hypothyroidism is iodine deficiency, but in most of the developed world and areas of adequate environmental iodine, cases are due to a combination of known and unknown causes.

- **Clinical Condition**

  Congenital hypothyroidism occurs when the thyroid gland fails to develop or function properly. In 80 to 85 % of cases, the thyroid gland is absent (athyrosis), severely reduced in size (hypoplastic), or abnormally located (ectopy). A hypoplastic gland may develop in the neck or even at the back of the tongue. These cases are classified as thyroid dysgenesis. In the remainder of cases, a normal-sized or enlarged thyroid gland (goiter) is present, but production of thyroid hormones is decreased or absent. Most of these cases occur when one of several steps in the hormone synthesis process is impaired; these cases are classified as thyroid dys-hormonogenesis, these account for 15% of the cases. Defects in thyroid hormone biosynthesis are familial, generally inherited in an autosomal recessive manner and include mutations in the genes coding for the sodium-iodide symporter, thyroid peroxidase, hydrogen peroxide generation [thyroid oxidase and dual oxidase maturation factors (THOX and DUOXA)], thyroglobulin (Tg), and iodotyrosine deiodinase. Uncommon causes of CH include defects in thyroid hormone transport (mutations in the gene for monocarboxylase transporter 8), metabolism (selenocysteine insertion sequence-binding protein 2), or resistance to thyroid hormone action (mutations in the thyroid hormone receptor). [2,4,5]

  Causes of transient CH are prematurity, iodine deficiency, maternal thyrotropin receptor blocking antibodies, maternal intake of anti-thyroid drugs, maternal or neonatal iodine exposure, loss of function mutations and hepatic hemangiomas).

- **Clinical Manifestations**

  Affected babies typically present normal weight and height. The first signs of CH is prolonged neonatal jaundice. Over time, undiagnosed children appear lethargic, with slow movements, hoarse cry, feeding difficulties, constipation, macroglossia, umbilical hernia, large anterior or posterior fontanels, hypotonia, dry skin, thinning hair and typical facies with saddle nose. Some newborns with dys-hormonogenesis present with a palpable goiter at birth. An X-ray of knee epiphyses may reveal delayed ossification, which reflects fetal hypothyroidism severity. Early treatment can prevent serious and permanent health problems, such as intellectual impairment, poor growth and hearing loss.

- **Treatment**

  The mainstay in the treatment of congenital hypothyroidism is early diagnosis and thyroid hormone replacement. Only levothyroxine (L-thyroxine) is recommended for treatment. It has been established as safe, effective, inexpensive, easily administered, and easily monitored. However, because of the potential for serious morbidity with inadequate treatment or overtreatment, primary physicians should consult a pediatric endocrinologist. Appropriate psychological, developmental, and educational evaluations should also be considered.

- **Prognosis**

  Most children born with congenital hypothyroidism and correctly treated with L-thyroxine grow and develop normally in all respects. Even most of those with athyreosis and undetectable T4 levels at birth develop with normal intelligence, although as a population academic performance tends to be below that of siblings and mild learning problems occur in some.

  Congenital hypothyroidism is the most common preventable cause of intellectual disability. Few treatments in the practice of medicine provide as large a benefit for as small an effort. [6]

- **Inheritance pattern**

  Most cases of congenital hypothyroidism are sporadic, which means they occur in people with no history of the disorder in their family. When inherited, the condition usually has an autosomal recessive inheritance pattern. This means the child need to receive 2 copies of mutated gene, one from the mother and other from the father to develop the condition. If the child receives one copy of the mutated gene, then the child will be carrier of the disorder. When both the parents are carriers of the diseased gene there is 25% chance with each pregnancy that they will have a child affected with the disorder. [6]

- **Few common symptoms**

  Lethargy, hoarse cry, cold sensitivity, poor feeding, large anterior or posterior fontanels, hypotonia, brittle hair [23]

- **Associated Congenital Malformations:**

  CH appears to be associated with an increased risk of congenital malformations (8-10 %).

e.g., Cardiac, Spiky hair, Cleft palate, Neurologic abnormalities, Genitourinary malformations. Congenital hypothyroidism is increased in patients with Down's Syndrome.

CONGENITAL ADRENAL HYPERPLASIA (CAH)

Congenital adrenal hyperplasia is an inherited disorder that results in decreased production of stress hormone cortisol and sometimes aldosterone, the salt-retaining hormone and increased levels of male hormones, causing development of male characteristics in females, and early puberty in both boys and girls.

CAH is one of the most common causes of preventable neonatal mortality and morbidity.

Congenital Adrenal Hyperplasia (CAH) is a group of inherited disorders affecting the adrenal glands. The most common form is 21-hydroxylase deficiency (21-OHD), which is inherited in severe or mild forms.

Non-Classical CAH. Non-classical(NCAH) (also known as Late-Onset CAH) is a variation of CAH that can begin to cause noticeable changes at any time from early childhood through early adulthood but is not immediately life-threatening. [6,8]

- **Clinical Condition**

  The adrenal glands produce cortisol, aldosterone and androgen. Congenital adrenal hyperplasia (CAH) impacts these three steroid hormones resulting in

  - lack of cortisol
  - lack of aldosterone.
  - too much androgen.

  Cortisol is needed to protect the body from the effects of illness or injury. Aldosterone hormone controls the amount of salt excreted in the urine. In the absence of this hormone, salt is lost uncontrollably, leading to dehydration and lack of salt. And the hormone androgen is present in both males and females. Androgen aids growth in childhood and is responsible for the puberty changes in woman. [25]

  Congenital adrenal hyperplasia results from mutations in the gene that codes for one of several enzymes responsible for making these steroid hormones in the adrenal glands. The most common enzyme to be affected is 21-hydroxylase also known as CYP21, which is a key regulator in the synthesis of the stress hormone cortisol. There is also an intermediate steroid precursor 17-Hydroxyprogesterone (17-OHP)

in the adrenal biosynthetic pathway from cholesterol to cortisol which is the substrate for steroid 21- hydroxylase. An inherited deficiency of 21-hydroxylase leads to greatly increased serum concentrations of 17-OHP. In this type of disorder, the adrenal glands fails to produce sufficient cortisol, instead produces excess amounts of androgen. In about two-thirds of affected individuals, the enzyme defect in the adrenal may also affect the production of the hormone aldosterone, which is responsible for retaining salt in the body. The decreased production of aldosterone can cause hypotension and hypoglycaemia, leading to neonatal death if not recognized and treated early. [11]

- **Clinical Manifestations**

  A fetus affected by congenital adrenal hyperplasia will produce excessive male hormones during development. If the fetus is female, this will result in virilisation of the external genitalia (i.e. they will look more masculine) and the female baby may be born with ambiguous genitalia (from the appearance of the external genitalia it is unclear if the baby is male or female sex). As both boys and girls with congenital adrenal hyperplasia have a deficiency of cortisol, they are likely to present in the first few days or weeks of life with an adrenal crisis unless treated with steroid replacement.

  During childhood, a normal healthy child switches off production of sex steroid hormones. However, if the infant has untreated congenital adrenal hyperplasia, they will produce excessive levels of male hormones from their adrenal glands. This will result in rapid growth of both boys and girls, with the additional effect of virilisation (development of male characteristics) of girls. Precocious (early) puberty, acne, hirsutism and subfertility are the commonest features of hyperandrogenism. In adult life, if the male hormones are not controlled, there might be multiple health problems, including infertility and the risk of developing obesity and hypertension due to exposure to excess steroids. [6,11]

  Common clinical presentation: Decreased activity, fatigue, Altered mental status, unresponsiveness, Poor feeding/weak suck, Dry mucous membranes, Hyperpigmentation, Abdominal pain, Vomiting, Hyponatremia, Hyperkalemia, Hypoglycemia, Metabolic acidosis, Hypothermia, Hypotension, Dehydration, Lack of weight gain.

- **Treatment**

  Glucocorticoid (GC) and mineralocorticoid (MC) replacement therapies are the mainstays of treatment of CAH. This can be achieved by administering the steroid

hormone therapy hydrocortisone during childhood. If a patient is in good control during adolescence taking hydrocortisone, he could continue this regimen. However, a minimum amount of long- acting GC may be added if adequate control is not achieved with hydrocortisone alone. In patients lacking aldosterone production, replacement with fludrocortisone is required to achieve normal salt balance. All classic CAH patients should receive fludrocortisone at diagnosis and during the 1st years of life. Levels of 17-hydroxyprogesterone, androstenedione and plasma renin activity are used to evaluate the adequacy of therapy in conjunction with clinical signs and symptoms of over-or under-treatment.

The exact treatment given will vary between patients depending on severity of their condition and the specific genetic mutation they have.

- **Prognosis**

  Good care and treatment results in a good prognosis and normal life expectancy. The main concerns in children are the preservation of fertility and healthy sexual function, and maintenance of general well being. This includes bone health and the assessment and management of cardiovascular disease risk. Optimal treatment of adults requires a multidisciplinary approach, including psychological support by specialists. This rare disease can be controlled through glucocorticoid treatment and, when needed, mineralocorticoid treatment. Patients who are adherent will maintain healthy, normal lives. Patients who are non-adherent will suffer many of the signs and symptoms of hyper-androgenemia. Poor adherence with medication can also lead to a potentially fatal addisonian crisis. [17]

- **Inheritance pattern**

  All forms of congenital adrenal hyperplasia (CAH) are inherited in an autosomal recessive manner. This means that to be affected, a person must have a mutation in both copies of the responsible gene in each cell. The parents of an affected person usually each carry one mutated copy of the gene and are referred to as carriers. Carriers typically do not show signs or symptoms of the condition. When two carriers of an autosomal recessive condition have children, each child has a 25% (1 in 4) risk to have the condition, a 50% (1 in 2) risk to be a carrier like each of the parents, and a 25% chance to not have the condition and not be a carrier.

GLUCOSE 6- PHOSPHATE DEHYDROGENASE DEFICIENCY (G6PD)

Glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency) is an X- linked inherited disorder of the blood. Most people with G6PD deficiency live normal lives and are

healthy. It is only when certain effects trigger a reaction in the red blood cells that the G6PD deficiency becomes a problem. This may occur when a person has an infection, fever, or when they take into their body a substance or chemical which the G6PD deficient red blood cell cannot cope with. When this occurs the red blood cells will react and get broken down and destroyed prematurely, the individual becomes anaemic and jaundiced. But, once the trigger factor is removed, the individual's health will return to normal.

- **Clinical Condition**

  G6PD is one of many enzymes that help the body process carbohydrates and turn them into energy. G6PD also protects red blood cells from potentially harmful byproducts that can accumulate when a person takes certain medications or when the body is fighting an infection.

  G6PD, the key enzyme in the oxidative pentose phosphate pathway, converts nicotinamide adenine dinucleotide phosphate (NADP+) into its reduced form, NADPH. NADPH is essential for protection against oxidative stress in erythrocytes.

  G6PD deficiency causes increased susceptibility of erythrocytes to H_2O_2 and other reactive oxygen species that can lead to hemolytic anemia. [21,22]

- **Clinical Manifestations**

  Babies with G6PD deficiency appear normal at birth. The peak incidence of neonatal jaundice occurs during the second or third day of life. The severity of the jaundice ranges from subclinical to levels compatible with kernicterus, a condition in infants characterized by damage to brain centers due to high levels of bilirubin. Acute hemolytic anemia usually begins within hours of an oxidative stress and ends when G6PD deficient erythrocytes have hemolyzed therefore, the severity of the anemia associated with these acute hemolytic episodes is proportionate to the deficiency of G6PD and oxidative stress. Viral and bacterial infections are the most common triggers, but many drugs and toxins can also precipitate hemolysis. The disorder, favism, in a G6PD deficient patient results from hemolysis secondary to the ingestion of fava beans, which contain beta-glycosides and naturally occurring oxidants. Although G6PD deficiency is thought to be benign, where enzyme levels are severely deficient there can be inadequate leukocyte function also. This results in chronic granulomatous disease.

- **Treatment:** Treatment for G6PD deficiency consists of removing the trigger that is causing symptoms. If the condition was triggered by an infection, then the underlying infection is treated accordingly. Any current medications that may be destroying red

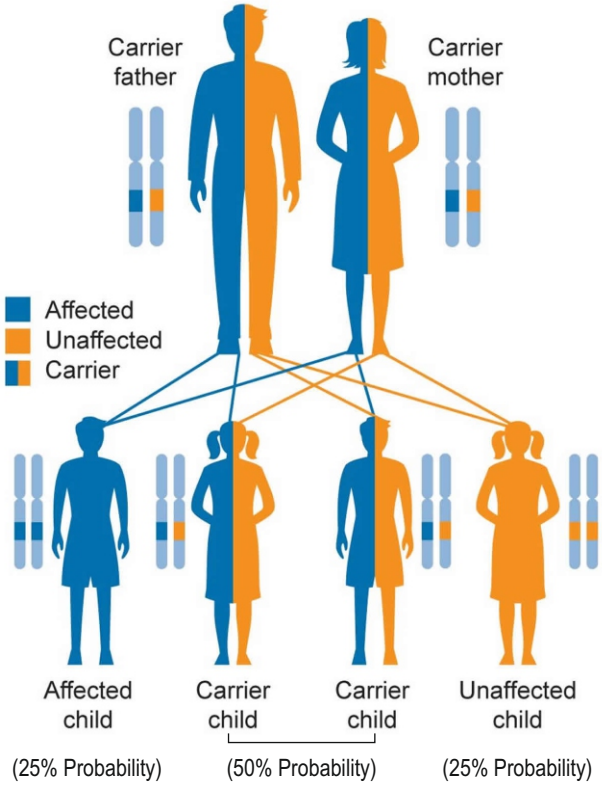
blood cells are also discontinued. In these cases, most people can recover from the condition on their own. If G6PD deficiency progresses to hemolytic anemia, more aggressive treatment may be required. This usually includes oxygen therapy and a blood transfusion to replenish oxygen and red blood cell levels. Though a cure does not exist for this condition, recognizing that kernicterus is a serious contributor to newborn mortality and morbidity in G6PD deficient babies, this should be enough of an impetus to start preventive measures. Without newborn screening to identify asymptomatic G6PD deficient newborns, these infants run a greater risk of unexpected hemolytic anemia if they unknowingly expose themselves to hemolytic triggers later in life. This goes to show that early institution of treatment does reduce or eliminate morbidity of the disease. Infants with prolonged neonatal jaundice as a result of G6PD deficiency should receive phototherapy with a bili light. Exchange transfusion may be necessary in cases of severe neonatal jaundice or hemolytic anemia caused by favism. The treatment for G6PD deficiency is avoidance. For the infant, this means avoidance of several medications prescribed for infections and illness such as. Antibiotics eg. Sulphanamides, Chloramphenicol Pain relievers eg. Paracetamol, Aspirin Malaria Drugs eg. Primaquine, Pentaquine Other Substances eg. Moth Balls, Fava (Broad) Beans including its pollen Prognosis Generally, the prognosis for G6PD-deficient patients is quite good. Most patients live relatively normal lives as long as they avoid triggers. [25]

- **Inheritance Pattern**

  G6PD deficiency is a genetically inherited condition and the gene for G6PD can be found on the X chromosome. Women have two X chromosomes (XX), one from each parent, whilst men have a single X chromosome from their mother and a male Y chromosome from their father (XY). As men have single X chromosome, G6PD affects more men than women. If a man inherits G6PD deficiency through the single X chromosome derived from his mother he will be G6PD deficient. A woman inherits two X chromosomes, one from each parent; therefore, if she inherits a normal X chromosome and a G6PD deficient X chromosome she will be an unaffected carrier because she still has a normal X chromosome. However, if she inherits two G6PD deficient X chromosomes she will be affected.

  G6PD deficiency is mainly found in Africa, Asia, and Mediterranean Europe, areas where malaria is endemic, or has been endemic. It has been suggested that the deficiency offers protection against malaria infection. The infection is usually not lethal in G6PD-deficient individuals possibly because Plasmodium can proliferate less efficiently in their erythrocytes.

Autosomal Recessive Inheritance



SUMMARY: NBS Core group of parameters: CH, CAH & G6PD

Congenital Disorders	Symptoms	Prognosis after treatment	Tests	Treatment and Management	Prevalence in India
Congenital hypothyroidism (CH)	Mental retardation, Poor Neurological Capabilities	Normal if treatment begins in the 1st month after birth	TSH and T4 Follow up tests IFTs and Radionuclide scans	Daily oral dose of thyroid hormone (thyroxine) Life-long treatment	10,400 cases annually[4] 1:130 newborns[5] Chennai-1/900[5] Cochin-3.9% Bangalore- 1:1042[5] Andhra Pradesh- 1:1700[6] Mumbai-1:2804[5] Kerala- 1:479[5] West Bengal- 7/600[13] Goa- 1:3440[14]
Congenital Adrenal Hyperplasia (CAH)	Ambiguity of genitalia, salt, Hyponatremia, Hypovolemia	Normal with medication	17- Hydroxy Progesterone (17-OHP) by Radioimmunoassay Or Tandem mass Followup/Confirmatory GC-MS (Gas spectrometry)	Hydrocortisone/ liquid prednisolone in infancy and childhood, and predisone or dexamethasone in adults	**In India overall 1:2575**[6] Chennai-1:2000[5] Delhi- 1:4208[5] Andhra Pradesh- 1:2600[6] Mumbai-118/70,590[12] Chandigarh-1:6813[5] Goa- 1:13800[14] Delhi- 38%[22] Kashmir- 1.4% in females[22] Kolkata- 1/1373[32]

Congenital Disorders	Symptoms	Prognosis after treatment	Tests	Treatment and Management	Prevalence in India
Glucose -6-Phosphate Dehydrogenase deficiency (G6 PD)	Anemia, Neonatal Jaundice, Anemia, Neonatal Jaundice, Hemolysis, Kernicterus	Normal with dietary and medical restrictions	Modified Formazan ring test method Or Modified Formazan ring test method Or ELISA/Enzyme assay	Treat infections. Transfusion if required Avoidance of drugs that cause hemolysis. Vaccination against hepatitis A and hepatitis B	Annually 3,40,000 cases in India[4] Chandigarh-0.89%[5] Delhi-2 %[5] Punjab- 3.9 %[5] Orissa-16.75 %[5] Mizoram-17.5 %[5] Surat- 22%[22] Andhra Pradesh-1:2200[6] West Bengal- 22/600[13] Kolkata- 1/1373[32]

CHALLENGES FOR NEWBORN SCREENING (NBS) IN THE DEVELOPING COUNTRIES

Newborn screening is an important public health measure aimed at early identification and management of affected newborns thereby lowering infant morbidity and mortality.

It is imperative that developing countries recognise the importance of Newborn screening as it has been proven through decades of experience that it saves thousands of babies from mental retardation, death and other serious complications.

Metabolic disorders have a high incidence in developing countries due to greater rate of consanguineous marriages. Newborn screening is recommended to reduce the burden of these disorders, as many metabolic disorders can be treated.

Guidelines from some developed countries recommend Newborn screening before discharge from hospital because of the high prevalence of certain endocrinopathies, metabolic errors etc., which if recognized later, contribute to significant morbidity.

However, neonates in most developing countries are not screened, the reason being

- Health policies have typically targeted mortality and infectious morbidities but not disabilities.
- Monetary and social burden i.e., cost factor to family and society, therefore clinicians are duty bound to reduce the burden.
- Though blood collection after 72 hours and within 7 days of life is the standard method of screening newborns for Hypothyroidism and metabolic disorders. In developing countries this practice has serious limitations due to high home delivery rate, early discharge from hospitals and cultural taboos related to newborns. In medical facilities with high turnover, babies are usually discharged within 24–48 h of delivery, thereby increasing the chances of inadvertently missing these babies for screening.
- Birthing centres are being spread out, therefore it is difficult to generate enough samples for laboratory testing.
- Countries with increased resources have recently shown how efficiently Tandem mass spectrometry (MS/MS) can be used for Newborn screening, but is a constraint for developing countries due to low resource settings, and competencies required to operate these automated systems.
- Huge capital investment and running costs required for NBS laboratory setup using traditional technologies.

- Delays in logistics, turn around time, therefore delay in reporting results and subsequently treatment.

So what is the solution, may be a need for POCT tests for Newborn screening which can be done near the patient with ease, requiring minimal skill, competence and staff. Usually POCTs (Bedside tests) in form of RDTs (Rapid ICTs) use a fraction of the blood sample, provide instant results therefore faster reporting and treatment initiation.

As National Neonatology Forum (NNF) in the year 2011, has recommended congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), and glucose-6-phosphate dehydrogenase (G6PD) deficiency as the core screening panel to Newborn screening in India.

Therefore developing RDTs for the above 3 disorders would be an appropriate choice of method for easier implementation of NBS in the low resource countries as it can lower financial and the social burden on the family and society as a whole.

Bibliography:

1. Bradford L. Therrell et al, Current status of newborn screening worldwide, Seminars in perinatology 39 (2015) 171 – 187, available online https://canpku.org/wp-content/uploads/2016/05/2015-Current-Status-of-Newborn-Screening-Worldwide.pdf.
2. I.C Verma, S.Bijarnia, The Burden of Genetic Disorders in India and a Framework for Community Control, Community Genet 2002, 5,192-196.
3. Eduardo Martı´nez-Morillo et.al., Challenges for Worldwide Harmonization of Newborn Screening Programs, Clinical Chemistry 62:5, 689–698 (2016).
4. Nazmin Fatima et al, “Screening of Inborn Errors of Metabolism- Use of Current and Future technology in Indian Scenario” International Journal of Health Sciences & Research, Vo.7, Issue 8, August 2017.
5. Ishwar C. Verma et.al “Newborn Screening: Need of the Hour in India” Indian Journal of Pediatrics, 82(1), December 2014.
6. A. Radha Rama Devi and S.M. Naushad “Newborn Screening in India” Indian Journal of Pediatrics, Volume 71, February 2004. pgs 157-160.
7. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian J Endocrinol Metab 2011;15:S78-81.
8. Rinku Shama, Birth Defects in India: Hidden Truth, Need For Urgent Attention, Indian Journal of Human Genetics, 2013, APR- JUN, 19 (2), 125-129.
9. Sudha Kohli et.al., Prenatal diagnosis of phenylketonuria, Indian J Med Res 122, November 2005, pp 400-403.

10. H.S Narayanan et al., Clinical and Genetic studies in cases of phenylketonuria (PKU) and a report of followup after dietary therapy, Indian Journal of Psychiatry, April 1988, 30 (2), 123-127.
11. Kannan Vaidyanathan et.al., Inborn Errors of Metabolism and Brain Involvement- 5 Years Experience from a Tertiary Care Centre in South India, www.interchopen.com.
12. Preeti Sharma et.al., Prevalence of Inborn Errors of Metabolism in Neonates., Journal of Clinical and Diagnostic Research. 2018 May, Vol-12(5): BC07-BC13.
13. Moushmi Lodh and Anand Kerketta, Inborn Errors of Metabolism in a tertiary care hospital of Eastern India, Indian Pediatrics, Vol. 50, 2013, pgs 1155-1156.
14. Seema P.S, Radhika D.S, Expanded newborn screening; Guiding principles, concerns and relevance, Muller Journal of Medical Science and Research, Vol.5, Issue 1, Jan-Jun 2014.
15. Ankur Singh et.al., Clinical Biochemical and Outcome Profile of Biotinidase deficient patients from tertiary care centre in North India, Journal of Clinical and Diagnostic Research, 2015 Dec, Vol -09(12); SC08-SC10.
16. Rita Christopher et.al., Maple Syrup disease: An Uncommon Cause for Neonatal Metabolic disorders, Indian Journal of Clinical Biochemistry (1999), 14 (2), 198-206.
17. Jailkhani R, Patil VS,et.al: Screening for Inborn Errors of Metabolism, Journal of Clinical and Diagnostic Research. 2008 Aug ;(2)952-958.
18. Roshan B. Colah, Malay B. Mukherjee, Snehal Martin & Kanjaksha Ghosh "Sickle cell disease in tribal populations in India" Indian Journal of Medical Research, 141, 509-515, May 2015.
19. David C Rees, Valentine A.M. Brousse. "Sickle cell disease: Status with particular reference to India" Indian Journal of Medical Research, Volume : 143, Issue 6, 675-677, 2016.
20. Moinak Sen Sharma et.al, Classical Galactosemia among Indian children; Presentation and outcome from a Pediatric gastroenterology center, Indian Pediatrics, Vol. 53, 2016, pgs 27-31.
21. Manisha Goyal et.al., Newborn Screening for G6PD Deficiency: A 2 year data from North India, Indian Journal of Public Health, Volume 59, Issue 2, April- June 2015, 145-148.
22. Seema Kapoor and Madhulika Kabra, Newborn Screening in India, Current Perspectives , Indian Pediatrics, Volume 47, March 17, 2010, 219-223.
23. Kapil U et.al., Prevalence of neonatal hypothyoroidism in Kangra Valley, Himachal Pradesh, European Journal of Clinical Nutrition, 2014 Jun, 748-9.
24. Unnikrishnan and Vyas, Congenital hypothyroidism, An Indian Perspective, Thyroid Research and Practice, Volume 14, Issue 3, September-December 2017.
25. S.K. Kabra, Cystic Fibrosis: Indian Experience, Indian Pediatrics, September 2002, 39, 813- 818.

26. Vishal Kapoor et.al., Carrier frequency of F508del mutation of cystic fibrosis in Indian population, Vol 5, Issue 1, January 2006, Pages 43-46.
27. Joanne V. Mel et.al., Performance properties of filter paper devices for whole blood collection, Bioanalysis, 2010, 2 (8), 1-7.
28. Usha P. Dave et.al., Chemical Diagnosis of Congenital Metabolic Disorders by Gas Chromatography/Mass Spectrometry (GC/MS) in India, IJHG I (3), 211-217 (2001).
29. Manjeet Kaur et.al, Inborn Errors of Amino Acid Metabolism in North India, Journal of Inherited Metabolic Disorders, 17(1994) 230-233.
30. CDC, Newborn Screening Quality Assurance Program Annual Summary Report, 2014, Vol 32.
31. S. Sachinananda Kamath, Newborn Screening in India, Indian Pediatrics, 2015,52,373-374.
32. Abhishek Kumar Tiwari et,al., A preliminary report on newborn screening of inborn metabolic disorders, Indian Journal of Child Health, Vol 6, Issue1, January 2019, 39-41.
33. N. Karthik Nagesh et.al., Newborn Screening, NNF Clinical Practice Guidelines,289-301, www.nnfpublication.org.

For the use of Registered Medical Practioners and Laboratories only

TULIP DIAGNOSTICS (P) LTD.

Gitanjali, Tulip Block, Dr. Rego Bagh, Alto Santacruz, Bambolim Complex ,
Post Office, Goa - 403 202. INDIA. Tel.: +91-832-2458546-51
E-mail: sales@tulipgroup.com Website: www.tulipgroup.com

Microxpress® Coral Clinical Systems BioShields orchid Viola