



*Meeting Report*

# Abstracts of Presentations Scheduled for the 10th ISNS-Asia Pacific Regional Meeting, Ulaanbataar, Mongolia, 24–26 August 2017

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Received: 7 August 2017; Accepted: 15 August 2017; Published: 15 August 2017

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## 1. Introduction

The International Society for Neonatal Screening (ISNS) recognises six different geographical regions. Of these six, the Asia-Pacific region is probably the one encompassing countries with the largest mutual economical differences which have led to large discrepancies in the way health care, including neonatal screening, has been developed in each of those countries.

ISNS aims to (co-)organise periodical conferences in the region and preferably in a less developed country to stimulate local professionals and policy makers to start or expand the neonatal screening programme. This 2017 conference has as theme “Racing to expand newborn screening in the Asia-Pacific region and Mongolia.

## 2. Invited Presentations

### *Plenary 1. The Challenges of Adding Disorders to Screening Panels*

R. Rodney Howell

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Newborn screening began with a single test, for a single condition, phenylketonuria. Such screening became virtually uniform more than 50 years ago in the USA. After this program was in place and quite successful other conditions were added to newborn screening panels which led to widespread variation across the regions, and growing need to establish careful plans for adding conditions to the newborn screening panels. The criteria originally presented by Wilson and Jungner for screening populations are widely used for choosing conditions to be added to newborn screening panels, and are being discussed elsewhere in this symposium.

In this presentation, I will discuss the Committee that was established by the United States Congress to generate an evidence-based newborn screening panel. The criteria of Wilson and Jungner were used as background information, but considered in the context of the genomic era. The Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC) was charged with reviewing conditions had been screened for in the newborn period and evaluating the data underlying these choices using the best data and science. From this work was developed the Recommended Uniform Screening Panel (RUSP) for all infants in the United States. The ACHDNC web-site (<https://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/>) provides detailed information about their deliberations. For many conditions reviewed for inclusion to the RUSP detailed evidence reviews are made publically available for review and very costly to replicate.

When a condition is considered for the RUSP, very large pilot studies are recommended, the Newborn Screening Translational Research Network (<https://www.nbstrn.org/about>) was established to assist with these studies and facilitate the development and assessment of new methods and technologies to improve early identification through newborn screening and other tasks for implementation. The costs of implementing newborn screening programs must always be considered (<https://www.marchofdimes.org>).

#### *Plenary 2. Newborn Screening in Europe*

J. Gerard Loeber

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Over the last 50 years almost all European countries have introduced neonatal screening as an important public health feature. Depending on health care structure, available funds, local politics, input from professional groups and the general public, this introduction has led to different approaches in the way the screening programmes have been set up, financed and governed. To get more information on these differences, in 2010 an online survey, commissioned by the EU, was compiled in which the whole screening programme was covered by a questionnaire. This survey covered the EU member states, (potential) candidate member states and EFTA countries, in total 38 countries. Results showed large variations in the panel of screened conditions, ranging from 0 to more than 30 conditions; specimen collection time after birth; screening methodology; and storage of residual specimens, varying from 1 to 1000 years. In addition, confirmatory diagnostics, treatment, and follow-up showed large discrepancies. In 2011 the project group provided a list of 60 recommendations to the EU Commission, but so far none of them have been taken up.

Recently the same colleagues were asked to update their data. Over the last five years, in some, mostly smaller, countries considerable changes have been implemented, mainly concerning the number of ms/ms detectable conditions. In contrast, in other mainly larger, countries very little has changed, if at all. Screening for SCID and CCHD, very much promoted and implemented in the USA is getting attention but so far only in pilot programmes in a few countries. In contrast to the US, in Europe national public health policies are either not at all, or only marginally, influenced by developments in neighbouring countries. It is therefore unlikely that NBS programmes in Europe will converge in the years to come.

#### *Plenary 3. Newborn Screening in the Asia Pacific Region*

Carmencita Padilla MD

Newborn Screening Reference Center, National Institutes of Health, MAHPS, University of the Philippines Manila

**Introduction:** The boundaries of the Asia Pacific Region are Mongolia on the North, New Zealand in the South, Pakistan in the West and the region includes all the lands and islands in between until the Americas in the East. Of the 138 million babies born in the world, almost half (67 million) are born in the Asia Pacific Region. Countries in the region vary widely in size, economic development and geography. There are many different languages, cultural sensitivities, and religions, each creating its own challenges in implementing NBS. There are currently more developing programs than developed programs within the region. Despite these challenges, NBS continues to grow throughout the region.

**Purpose:** This session will provide: (1) an overview of the development of newborn screening programs in Asia; (2) recent developments; and (3) continuing challenges on country implementation.

**Materials and Methods:** Country newborn screening programs were reviewed in 2015 to identify developments in both developed and developing countries in terms of absence/presence of a program, screening coverage, conditions included (routine and pilot), challenges and plans.

Results: There are 4 groups of countries: (1) some NBS programs within the region have included expanded newborn metabolic screening as a routine panel for all newborns; (2) some NBS programs offer expanded newborn metabolic screening as an option to families at an extra cost; (3) some NBS programs offer only CH plus another disorder; and (4) some have no NBS programs yet.

Conclusions: Developed and developing countries have continuing challenges. Developed programs continue to look for new conditions to add to the panel and for some, increasing coverage of expanded newborn screening. The challenges for developing programs range from starting the program, strengthening the infrastructure, increasing conditions in the panel, introducing expanded newborn screening, laboratory QA issues, follow up and management of patients diagnosed by the program.

#### *Plenary 4. Newborn Screening Worldwide: Molecular Testing Is Becoming Part of Routine Newborn Screening*

Bradford L. Therrell

U.S. National Newborn Screening and Global Resource Center, Austin, TX, USA

Introduction: Newborn bloodspot screening (NBS) includes various tests that can occur within hours of birth and have the potential for preventing severe health problems, including death. NBS has evolved from a simple blood or urine screening test to a more comprehensive and complex screening system capable of detecting over 50 different conditions. Molecular testing using dried blood spot specimens was first demonstrated as a viable 2nd-tier NBS technique for both sickle cell diseases and cystic fibrosis in the 1980s. Since that time, molecular testing has slowly expanded in NBS programs until now it is routine in many screening programs including use as a primary screening method for SCID.

Purpose: This presentation: (1) provides a brief overview of NBS activities worldwide; (2) reviews the origins of molecular testing as part of NBS; and (3) assesses molecular testing activities in NBS today.

Materials and Methods: A series of reports described NBS activities in 5 regions of the world in 2007. These reports were updated in 2015 [Brad Therrell and John Adams (North America), Carmencita Padilla (Asia-Pacific), Gerard Loeber (Europe), Issam Khneisser and Amal Saadallah, Middle East/North Africa, Gustavo Borrajo (Latin America)]. These reports have been reviewed to assess trends in adding molecular testing.

Results: NBS for one or more conditions now covers slightly more than one-third of the world's newborns. In more developed programs, molecular testing protocols are included either as 2nd-tier (cystic fibrosis and sickle cell diseases) or as primary screening (SCID).

Conclusions: Institutionalizing and sustaining NBS presents a formidable challenge, particularly in developing economies. While developing programs struggle to build infrastructure and implement screening for the basic screenable conditions, developed programs are moving forward with molecular testing strategies. Future expansion of NBS will continue to include molecular testing as a screening practice.

#### *Plenary 5. Screening, Diagnosis and Management of Endocrine Disorders with Limited Resources*

Paul Hofman<sup>1,2</sup>

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Congenital hypothyroidism and congenital adrenal hyperplasia (CAH) are endocrine conditions that, if not recognised and treated appropriately, cause irreversible complications or death in early infancy. The case for neonatal screening for congenital hypothyroidism is irrefutable with progressive and permanent developmental delay resulting from delayed diagnosis. Most newborn screening programs use TSH as the main or only marker to detect primary hypothyroidism. While there is currently debate as to the lower limit for TSH reporting in developed countries, the role of screening in

developing countries is to identify cases that are likely to have clinically important hypothyroidism. Thus using higher reporting levels reduces false positives and ensures resources are focussed on the most severe cases. In situations where there is resource constraint other factors are relevant including the timing of the blood sample (for instance cord or a 2 day heel prick), the population being screened (e.g., whole population, larger cities or specific hospitals), the ability to contact families with potentially affected children and the availability of post natal testing and medications that can modify the later outcomes. These issues are discussed with relationship not only to congenital hypothyroidism but also the severer, salt wasting forms of CAH. With CAH there remains more controversy about the benefits of screening, both in terms of the diagnostic positive predictive value of the tests used (usually 17 hydroxy progesterone assays) and the need for therapy before symptoms occur. However over the past 20 years the majority of developed countries have opted for CAH screening. Paradigms for both CAH and congenital hypothyroidism will be discussed that have been used with varying success in other countries and a range of approaches will be suggested for developing countries. Notably for any program to succeed it must have local medical and nursing advocates as well as a commitment by the government to provide the necessary financial support needed to provide an ongoing program.

#### *Plenary 6. Reducing False Positive Results for Endocrine Disorders*

Toni Torresani

Zürich, Switzerland

The issue of false positive results in neonatal screening has always been a debated topic. The anxiety of the parents arising from such false positive results, has always been the driving reason for trying to find ways to effectively reduce them. Unfortunately, quite often in these discussions the notion that a neonatal screening test simply indicates that a baby might have a condition, is overlooked. Furthermore, when different programs compare their performances, the different conditions for sample collection, transport and analysis are not taken into consideration. Whether a neonatal screening result can be classified as within or outside limit, is not only dependent from the above-mentioned conditions, but also from several clinical conditions like maturity, time of sample collection, general health condition of the baby and sometimes also from the mother.

Taking into account all or some of the above-mentioned conditions when interpreting neonatal screening results, will help in reducing substantially the number of false positives, in almost all tests performed. Particular measures that can be applied to neonatal screening of endocrine diseases, congenital hypothyroidism (CH) and congenital adrenal hyperplasia (CAH), will be presented and discussed.

#### *Plenary 7. A New CLSI Guideline on Hemoglobinopathy Newborn Screening and the Results of Two Decades of Newborn Screening for Sickle Hemoglobinopathies in the United States*

Bradford L. Therrell

U.S. National Newborn Screening and Global Resource Center, Austin, TX, USA

**Introduction:** Hemoglobinopathy newborn screening (NBS) has existed since the 1970s, primarily for detection of sickle cell diseases (SCD) in the U.S. and the thalassemias in other regions of the world, including Asia. There is limited knowledge of the overall prevalence of SCD (SS-disease, SC-disease, S beta-thalassemia) and sickle carriers in the U.S., and thalassemias elsewhere. A source for international guidance for NBS programs does not exist.

**Purpose:** The Clinical Laboratory Standards Institute (CLSI) is finalizing a new international guideline for hemoglobinopathy NBS that outlines laboratory and other system issues with appropriate recommendations for optimal NBS implementation. Additionally, the outcome of two decades of screening data for sickle hemoglobinopathies in the U.S. is now available for review.

**Materials and Methods:** A defined protocol for preparing CLSI guidelines was followed. U.S. SCD data were collected from program reports to the national data system from 1990 to 2010.

**Results:** The CLSI Guideline is now in final review. The U.S. 20-years data revealed that there were 39,422 confirmed SCD cases among 76,527,627 newborns screened (1:1,941) and 1,107,875 laboratory reports of probable sickle carriers among 73,951,175 newborns screened (1:67). SCD was most prevalent in the District of Columbia (1:437) followed by Mississippi (1:683) and South Carolina (1:771). Likewise, sickle cell carrier prevalence was highest in the District of Columbia (1:22), Mississippi (1:26) and South Carolina (1:31).

**Conclusions:** An international guideline on NBS hemoglobinopathy screening will be helpful as a resource for harmonizing and improving NBS activities worldwide. The U.S. data will assist in: (1) planning for health services delivery; (2) providing data for researchers; (3) aiding in tracking health outcome trends; and (4) assessing sickle gene prevalence in the newborn population.

#### *Plenary 8. Screening and Diagnosis of Metabolic Disorders*

Veronica Wiley

NSW Newborn Screening Programme, SYDNEY, Australia

Electrospray ionisation tandem mass spectrometry has been used in NSW since 1998 and has now been incorporated in many newborn screening programs worldwide. There have been many changes in methodology to prospectively screen newborns for inborn errors of amino acids, fatty acid oxidation and organic acidurias. However despite many attempts to harmonise its introduction, it remains the responsibility of each program to determine which disorders are screened, what sample to use, and what is acceptable performance.

For each analyte tested there is sample, analytical and interpretative considerations. Sample aspects include the optimal time of collection after birth, the effect of feed status and gestational age. Analytical considerations include the instrumentation, sample preparation, establishing action limits, normal percentiles and expected results for proven positives as well as appropriate quality assurance protocols. To optimise the performance metrics of resample rate, sensitivity, specificity and positive predictive value further testing can be performed on the initial sample and this include ratios of analytes or second tier testing including DNA variant analysis.

Screening is however not diagnostic and further samples need to be requested from those suspected of having a disorder. The samples required depend on the suspected disorder including the urgency required to provide treatment. Samples required for diagnostic testing by biochemical genetics may be urine, plasma, CSF, skin or other biopsy specimens.

Using various MSMS protocols since 1998, we have screened samples collected at 48–72 h of age from nearly 2 million babies, request further samples from 0.15%, and detect a disorder in 1:2691 babies with a sensitivity, specificity and positive predictive value which are currently 99%, 99.9% and 25% respectively.

The use of tandem mass spectrometry in newborn screening is expanding to include many other disorders. The challenge remains how to optimise protocols for each specific disorder.

#### *Plenary 9. Deciding on Disorders for Inclusion in a Newborn Screening Programme*

J. Gerard Loeber

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This topic starts with the assumption that there is an infrastructure for newborn screening in place and that funding is available. The disorder of first choice is congenital hypothyroidism because it is very prevalent, irrespective of ethnicity, the screening and diagnostic methodology is relatively simple and the treatment is relatively cheap. For decision on further disorders the framework of the Wilson and Jungner criteria is very valuable, even after 50 years since its inception. Important

considerations are: is the disorder in your jurisdiction an important health problem AND is the test acceptable for the population from cultural/ethical perspective (unintentional findings; carrier status; mild and late-onset forms).

If the answer to both questions is affirmative, then the cost of the existing situation (detection by clinical symptoms) should be compared with the cost of screening; this is very much dependent on the prevalence of the disorder. If the cost-benefit ratio is positive then it should be checked if the existing facilities are adequate to also cover this disorder when added to the panel: adequate test method, clinical confirmation, follow-up and treatment, information and education of professionals and general public, informed consent especially when carriers will be detected.

Disorders should NOT be added, just because the apparatus is capable of picking it up or because a clinician happens to have one patient in his practice and has become interested in that disorder or because a politician has a family member suffering from it.

References: Cornel, M.C.; Rigter, T.; Weinreich, S.S.; Burgard, P.; Hoffmann, G.F.; Lindner, M.; Gerard, L.J.; Rupp, K.; Taruscio, D.; Vittozzi, L. A framework to start the debate on neonatal screening policies in the EU: An Expert Opinion Document. *Eur. J. Hum. Genet.* **2014**, *22*, 12.

#### *Plenary 10. Strategies in Overcoming Geographic Obstacles*

Ma Elouisa Reyes

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University of the Philippines Manila

**Introduction:** The Philippine newborn screening program (NBS) started in 24 hospitals in Metro Manila in 1996. It was integrated in the public health delivery system with the passage of the Newborn Screening Law in 2004. Among the major challenges of the program at the beginning was the 7000+ islands of the country. The Philippines is made up of 3 groups of islands subdivided into 18 political regions and 81 provinces (a mix of rural and urban cities).

**Purpose:** This session will provide: (1) challenges in the implementation of the program (2) strategies to overcoming geographic obstacles.

**Materials and Methods:** Practices through existing IEC materials/Philippine Assessment Tools/Technical Reports since 2005 were reviewed to identify challenges and strategies made to resolve geographic concerns.

**Results:** Among the challenges in implementation were: (1) initially, only the big cities were accessible; most rural areas are either island-based or mountainous, (2) home deliveries making NBS difficult; and (3) schedules of airports and ports initially limited prompt submission to laboratories; and (4) no available courier service in most rural areas. Among the strategies employed were: (1) strategic location of the 6 newborn screening laboratories; (2) engagement of health workers to go to houses for sample collection; (3) strategic location of 14 follow-up and management centers (continuity clinics); (4) coordination with the Department of Health (DOH) offices and the local government units for the hard-to-reach areas for transmission of samples and results, and follow up of positive cases; (5) engagement with the private sector (i.e., courier and transportation companies).

**Conclusions:** The Philippine terrain is a continuing challenge to the program. It lessened through the years as more strategies in the national and local levels are developed and implemented; and a close coordination between NBS laboratories and DOH offices to resolve operations in geographically challenged areas.

#### *Plenary 11. Building National Qa Programs In China—Laboratory and Non-Laboratory Aspects*

Zhengyan Zhao

Children's Hospital of Zhejiang University School of medicine, Department of genetic and metabolism, Hangzhou, China, Peoples Republic

The China Newborn Screening Program started in 1981 covering on average 17 million newborns annually. As a public health project, the screening rate has reached 97% in 2016. The newborn screening disorders started from the initial two diseases (CH, PKU) to G6PD and CAH and then expanded to 32 diseases by tandem mass spectrometry. Gradually, related laws and regulations are legislated and improved, quality control management systems are formed, guidelines are released, and affected babies in undeveloped areas are aided. The Chinese newborn screening quality control systems are manipulated in national, province, city and county levels. The NCCL organizes the quality assurance program, including PT, QC, information, quality index, on-site inspection and training summary. In recent years, information management promoted the connection with all related units of newborn screening to ensure the effectiveness of recall and follow-up, and achieve the integrated management of screening, diagnosis and treatment.

### 3. Oral Presentations

#### *O1. Screening for Hemoglobin Disorders: Philippine Experience*

Carmencita Padilla MD

Newborn Screening Reference Center, National Institutes of Health, MAHPS, University of the Philippines Manila, Philippines

**Introduction:** Newborn screening (NBS) in the Philippines began as a small pilot program in Manila in 1996 and became a nationwide program in 2004 by law requiring that NBS be offered to all newborns, supported by national health insurance. As screening for 6 conditions became routine in all hospitals, expansion to additional disorders was the next challenge.

**Purpose:** This session will share the development of NBS for hemoglobin disorders: preparations, algorithms, initial data, and challenges in implementation.

**Methods and Methods:** The Philippine program was fortunate to secure data on 110,000 Filipino newborns in the California Newborn Screening Program. The Philippine Department of Health accepted the data as basis of the expanded newborn screening (eNBS). Workshops with key players (NBS laboratory staff and follow up teams, geneticists, and pediatric hematologists) were conducted by Bradford Therrell of Newborn Screening and Genetics Resource Center, USA; David Millington, metabolic geneticist of Duke University (for metabolic disorders); and Carolyn Hoppe, pediatric hematologist of UCSF Benioff Children's Hospital Oakland (for hemoglobin disorders). After preparations of the laboratory (screening and confirmatory), short term and long term follow-up teams, the Philippines started eNBS on December 2014. Patients now are given 2 options: Option 1 fully funded by the national health newborn screening—screening for 6 conditions such as congenital hypothyroidism, congenital adrenal hyperplasia, phenylketonuria, galactosemia, maple syrup urine disease and G6PD deficiency; and Option 2 partially funded eNBS for conditions detectable with tandem mass spectrometry, hemoglobinopathies, cystic fibrosis and biotinidase deficiency.

**Results:** NBS results for hemoglobin disorders of the first 300,000 newborns will be presented.

**Conclusion:** New challenges faced by the Philippine program with the inclusion of hemoglobin disorders are: training of teams, development of algorithms for both laboratory and clinical follow up, engagement of hematologists for long term care, and cost of treatment.

#### *O2. Pilot Newborn Screening in Nepal*

Arti Pandey

Kathmandu Medical College and Teaching Hospital, Dept. Biochemistry, Kathmandu, Nepal

**Introduction:** While newborn screening (NBS) has been established in many countries, already 50 years ago, there are still countries where no NBS is so far established. We report on the first preliminary results of a pilot NBS program in Nepal.

**Purpose:** The main purpose of this pilot was to show the feasibility of NBS in Nepal, and probably get first positive results.

**Materials and Methods:** The pilot study was established between, Kathmandu Medical College and Teaching Hospital, and the University Children's Hospital in Zurich, which was approved by the Nepal Health Research Council (NHRC). 5000 dried blood samples (DBS) were collected in Nepal and sent to Zurich, where they were tested according to the Swiss NBS program.

**Results:** Within these 5000 samples 1 child with congenital hypothyroidism, and 1 child with Cystic Fibrosis was detected. Median values and relevant percentiles were comparable to the values from Swiss NBS program for most analytes. However, enzyme activities (GALT and Biotinidase) were significantly lower, most probably due to the time delay and transportation.

**Conclusion:** The pilot program showed the feasibility of NBS in Kathmandu region. Since the total number of samples was rather small, we are planning to extend this pilot to a further 10,000 samples, to gather more data on the more rare inborn errors of metabolism.

### *O3. Pilot Scheme for the Establishment of a Neonatal Screening Program in the Democratic Republic of Laos*

Thomas Hoehn, Zoltan Lukacs and Saysanasongkham Bounnack

University Hospital Duesseldorf, General Pediatrics, Neonatology and Pediatric Cardiology, Duesseldorf, Germany

**Background:** Neonatal screening programs have been established and are in use in many countries worldwide. Countries without any established neonatal screening programs in 2009 included Nepal, Cambodia, Laos, and the Pacific Island nations.

**Aim:** To show the feasibility of neonatal screening in the urban setting of Vientiane, capital of Laos. Furthermore to expand regular screening to other cities within Laos and to offer ongoing teaching to local pediatricians with respect to further follow-up of infants with diagnosed inborn errors of metabolism.

**Methods:** Initial participants were the large maternity hospitals within the city of Vientiane (Mahosot Hospital (2.500 deliveries p.a.), Sethathirath Hospital (2.000 deliveries p.a.), Friendship Hospital (700 deliveries p.a.), and Mother and Child Health Hospital (3.500 deliveries p.a.)). Samples were taken immediately prior to hospital discharge and once weekly air-shipped to a German screening laboratory.

**Results:** 11.362 samples of newborn infants have been examined. The rate of retests was above European average due to very early discharge policies in Laotian maternity hospitals. Confirmed cases of neonatal congenital diseases include two infants with hypothyroidism and one infant with congenital adrenal hyperplasia without salt-loss. All three infant received early therapy and are currently doing well.

**Conclusions:** Even in a very low resource setting as in Laos the establishment of a neonatal screening program appears to be feasible. Further challenges include the expansion to the rest of the country and the on-site establishment of measurements within Laos.

### *O4. 10 Years for the Neonatal Screening on Phenylketonuria in Kazakhstan*

D.N. Salimbayeva

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**Background.** Phenylketonuria (PKU; OMIM: #261,600) is an inborn error of phenylalanine metabolism. The effective method of the early diagnostics and prophylactics of PKU are a neonatal screening. Since 2007 year the national program of neonatal screening for phenylketonuria was introduced in Kazakhstan.

**Methods.** In Kazakhstan we use for the neonatal screening of PKU immunofluorescence method with standard kits (Neonatal Phenylalanine kit, PerkinElmer).



**Results.** In 2007–2016 years more than 2.5 million newborns were studied for PKU. Since 2007 year the middle coverage of neonatal screening in Kazakhstan increased from 51.9% to 81.0%. Kazakhstan has 16 regions and 2 megalopolis. The coverage of PKU in these regions is different, but in 9 regions over 95%. The effective state program of neonatal screening in Kazakhstan allowed to decrease treatment initiation by PKU until <15 days of life. The middle rate of PKU in Kazakhstan is 1:26,893 newborns. The rate in regions varies significantly from 1:65,310 in Astana to 1:5020 and 1:5087 in Kostanay and Aktau regions. This variability depends on ethnic structure of populations in these regions. In regions with more European population (Russian, Ukraine and others) the rate of PKU is bigger than in regions with Kazakhs. The PKU patients Uighurs and Dungan, were only in South Regions of Kazakhstan. The program of neonatal screening revealed 105 newborns with PKU. The patients with PKU in Kazakhstan have different nationality. From them Russians 48.1%, Kazakhs 34.2%, Uighurs 5.1%, Ukraine 3.8% and others 9.2%.

**Discussion.** Consequently, introduce neonatal screening allow to opportunely reveal and diagnose diseases before clinic manifestation. The feature of Kazakhstan's population and ethnic difference are important for molecular genetic diagnostics.

#### *O5. Long-Term Follow-Up of Tetrahydrobiopterin (BH4) Therapy in Patients with BH4 Deficiency and bh4 Responsive PKU (BPKU) in Japan*

Saki Kasuga, Haruo Shintaku, Tomoko Sakaguchi, Takashi Hamazaki, Takao Hoshina, Kazuyoshi Tomita and Noriatsu Hikita

Osaka City University Graduate School of Medicine, Pediatrics, Osaka, Japan

**Introduction:** Nationwide NBS for PKU was initiated in Japan in 1977, and the differential diagnosis for tetrahydrobiopterin (BH4) deficiency was started in 1982. Sapropterin dihydrochloride granules 2.5% (Biopten<sup>®</sup>, Daiichi Sankyo, Japan) was approved in 1992 for treatment of BH4 deficiency and in 2008 for treatment of BH4 responsive PKU (BPKU) in Japan. A longitudinal follow-up study is being conducted of all patients with BH4 deficiency and BPKU throughout Japan. In this analysis, we evaluated the efficacy and safety of BH4 therapy initiated in patients under 4 years old.

**Patients and Methods:** BH4 therapy started in patients of less than 4 years of age was 27 (PTPS:25 and DHPR:2) out of 40 in BH4 deficiency and 32 out of 61 in BPKU. Patients with BH4 deficiency were treated with BH4 plus L-dopa and 5-HTP. We conducted a longitudinal retrospective study that examined plasma phenylalanine (Phe) levels and adverse reactions.

**Results:** The plasma Phe value was maintained within a favourable range in all patients in whom BH4 therapy was started before 4 years of age. No considerable abnormal behaviour related to nerve disorders was reported. No unwarranted side effects were reported throughout the long-term course of treatment.

**Discussion and Conclusions:** BH4 therapy in patients with BH4 deficiency and BPKU is highly effective at maintaining serum phenylalanine levels within a normal control range and provides excellent safety throughout life with no unwarranted side effects.

#### *O6. Neurological Outcome of Adult PKU Patients Detected by NBS in Japan*

Kenji Yamada, Kikumaro Aoki, Kazunori Yokoyama, Hironori Kobayashi, Yuki Hasegawa, Go Tajima, Haruo Shintaku, Takeshi Taketani and Seiji Yamaguchi

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**Introduction** Nationwide newborn screening (NBS) for metabolic diseases including phenylketonuria (PKU) was initiated in Japan since 1977, 39 years ago. To investigate the effect and problem issues of NBS, we surveyed neurological outcomes of Japanese PKU patients in adulthood.

**Subjects and Methods** for 85 PKU patients aged 20 or above, we performed the questionnaire about clinical characteristics including mentality, education, working, and therapy condition.

Results of the 85 patients, 16 were detected before initiation of NBS (age of >39 years, pre-NBS group), while 69 patients were detected after starting NBS (age of <38 years, post-NBS group). 8 of the 69 post-NBS patients had mental retardation (MR) and 7 of them presented borderline MR. By contrast, 10 of the 16 pre-NBS patients had MR, 6 of which showed severer. 60 of 66 patients graduated from university (31 cases), junior vocational college (12), or high school (17), while 6 cases finished school for handicapped children. 5 of the 6 cases did not undergo NBS. 8 of 9 patients who were incapable to work were pre-NBS group.

**Discussion and Conclusions** Our study showed that the neurological outcomes were obviously improved by NBS. The severity of MR in the post-NBS group was mild. Many of the patients with MR in the post-NBS group had ever quitted the dietary therapy due to diet difficulty, economic problems, or insufficient education by medical staffs. Further, in the patients aged from 35 to 38 of post-NBS group, mental problems were relatively frequent. This is because it had been considered that dietary therapy after school ages might not be necessary until early in 1980s.

In conclusion, NBS is apparently effective, but continuous dietary therapy is essential to prevent neurological impairments.

#### *O7. Some Results of First Newborn Hearing Screening in Mongolia*

Ch. Saruul and B. Delgermaa

NCMCH, Mongolia

**Study purpose:** Our study aimed to detect hearing loss and deafness among infants as early as possible with the automated auditory brainstem response.

**Study material, and methodology:** We have started the screening from December 2012 and by the second quarter of 2016 been able to include total of 9379 newborns. We tested the automated auditory brainstem response of the infants in their 1–3 days of birth, using Maico MB11 Beraphone machine (German) according to the Joint Committee on Hearing Screening guidance Joint Committee on Hearing Screening. We assessed the test result of “pass” as “normal hearing”, “refer” as to rescreen and tested again after 1 month. For the second occurrence of “refer” result, we have dispatched to the following specialized audiologic tests. We have done ABR test for the infants when they reached 3 months.

**Study results:** Our study was the first in Mongolia and 8023 of the 9379 newborns participated in the hearing screening tested with the result of “pass” and 1356 newborns with “refer”. For the rescreen test, 447 of the 546 infants resulted with “pass” and 99 infants with “refer”. For those infants, we did specialized audiologic tests in their 3, 6, 9 months, and result of 6 children (12 ears) having bilateral profound hearing loss, 3 children (6 ears) having bilateral mixed profound hearing loss. First refer rate was 14.4%. Second refer rate was 4.5%. Follow rate was 40.2%. We are preparing children with bilateral profound hearing loss of 0–3 years old for the follow up CI surgery.

**Conclusion:** In Mongolia, the data of infant hearing difficulties and prevalence of deafness is lacking. Although a newborn hearing screening program has been approved in 2014 and brought opportunities to conduct universal neonatal hearing screening program, its only available at NCMCH. Our study’s preliminary result shows a similar pattern (1.1–1.3 ear deafness in every 1000 birth. Lenarz et al., 2008) of 9 cases of bilateral profound hearing loss out of 9379 newborns. Small population on the remote locations, high birth percentage, short period in hospital after birth, lack of technology and human resources, and other factors result in higher level of referral rate newborn hearing screening and lower level of follow up and confirmation rate referrals in Mongolia.

#### *O8. Implementation of Newborn Pulse Oximetry Screening for Chd in Rural China*

Annamarie Saarinen, Jia Chen MD and Jing Yu

Newborn Foundation, Newborn Screening | Research Institute, North Oaks, USA

The concept of newborn screening for congenital heart disease (CHD) developed slowly from small studies between 2002 and 2009 to larger population based studies in Europe and the U.S. These studies and associated advocacy provided key data driving the US Department of Health and Human Services to formally add Critical Congenital Heart Disease (CCHD) screening to the Recommended Uniform Screening Panel (RUSP) in the United States in 2011. Today, at least 48 international countries have either implemented or are in the process of evaluating newborn screening using pulse oximetry. At least 10 countries have recommended or require screening. Challenges remain however, particularly in low-resource health settings. The presentation will focus on implementation methods and results from screening more than 20,000 well-appearing newborns across 18 birth facilities in rural Sichuan Province China, utilizing a low-cost, mobile-phone pulse oximeter to measure blood-oxygen saturations at two points ; 12 and 48 h after birth. Overall incidence of congenital heart disease (CHD) in the region is higher than the national average at 11.74% (vs. 6.75%), with 20~30% of CHD patients dying within the first year. In addition to the data on CHD detection, the presentation will show that more than 50% of failed screens resulted in a diagnosis of previously unrecognized neonatal pneumonia. Neonatal infection (sepsis and pneumonia) rank among the most common causes of newborn mortality, accounting for more than a quarter of newborn deaths each year in China and in many developing nations globally. The sensitivity and specificity of screening in identifying non-target conditions combined with reducing missed or delayed diagnosis of congenital heart diseases make this an increasingly high impact screen for babies in under-resourced settings. The project deployed a train-the-trainer implementation model supported by public health and clinical leadership and robust data collection to identify and improve gaps in follow-up diagnostics, referral pathways and treatment infrastructure.

#### *O9. The Prevalence of Fetal Congenital Heart Defect*

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**Background:** Congenital heart defect (CHD), the most common type of birth defects, affects nearly 10 per 1000 live births, or 13–35 per 1000 fetuses. Therefore, fetal congenital heart defect incidence is higher than congenital heart defect among neonates. The combined heart defects and heart defect with chromosomal anomalies are leading causes of early fetal mortality and stillbirth. Mothers with CHD have an increased or 10–14% risk of delivering a child with cardiac anomaly. If the father has CHD, the risk is lower and approximately is 2–3%. Maternal medication and alcohol exposure and smoking have been identified as potential cardiac teratogens (anticonvulsants—1.8%, corticosteroids and anti-inflammatory drugs—1–2%). Some maternal conditions also carry an inherent risk to fetus.

**Study objective:** To diagnose fetal congenital heart defect by fetal echocardiography and determine its prevalence and type.

**Materials and Methods:** We used hospital based cross sectional study design and analyzed pregnant women in 18–36 gestational week by fetal ultrasonography dividing women into two groups: with no risk as control group and with more than one risk as case group.

**Results:** We involved 822 pregnant women with 463 in control group and 359 in case group. CHD was diagnosed in 125 fetuses in total with 25 (5.4%) in control and 100 (27.8%) in case group. In 60 fetuses (48%) minor CHD was revealed comparing to 65 (52%) in which the major heart defect was diagnosed by fetal echocardiography. Maternal exposure to medication during first-trimester (OR = 14,  $p = 0.001$ ), maternal illnesses (OR = 3.7,  $p = 0.001$ ) and late pregnancy (OR = 3.4,  $p = 0.001$ ) were revealed as risk factors to affect the fetal heart.

**Conclusion:** Fetal heart defect prevalence was 15.2%, which is similar to the researchers in other countries. Fetal heart defect occurred in every fourth fetus (27.8%). 52% of these defects are risky and complicated CHD.

*O10. A Novel Method for Inclusion of All Urea Cycle Disorders into Newborn Screening*

Ralph Fingerhut, Mia Halme and Johannes Häberle

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**Introduction:** The inclusion of urea cycle disorders (UCD) into newborn screening (NBS) is highly desirable but hampered by the lack of specific markers. Exceptions are citrulline and argininosuccinate (ASA) for detection of citrullinemia and argininosuccinic aciduria, respectively. So far, the common feature of all UCDS, hyperammonemia, is not directly detectable in dried blood spots (DBS). The quantification of secondary elevations of glutamine was not feasible based on the assumption of instable glutamine in DBS.

**Methods:** The NeoMass AAAC test kit was used together with a Waters Xevo TQD tandem mass spectrometer (TMS) and a Waters Aquity H-Class UPLC. Aim was a reliable method for the simultaneous detection of lysine and glutamine from DBS in multiple reaction monitoring with a second-tier UPLC-method for the separation and specific quantification of glutamine. We combined this newly developed method with the measurement of all relevant amino acids (arginine, ASA, citrulline, ornithine, and proline), N-acetyl-glutamate, and orotic acid.

**Results:** 388 anonymized samples from healthy newborns were tested. The median and 99th centile for the sum of glutamine and lysine were 212 and 958  $\mu\text{M}$ , respectively. 103 samples were additionally separated by UPLC. Median and 99th centile were: 65 and 534  $\mu\text{M}$  (lysine), and 507 and 899  $\mu\text{M}$  (glutamine). In addition we have analysed samples from patients with OTC, citrullinaemia, CPS-1, and hyperlysinaemia. Orotic acid was only elevated in the samples from the OTC patient.

**Conclusions:** We describe a reliable and sensitive method for the detection of all UCDS by TMS-NBS. The next step will be a prospective study with DBS samples from patients with hyperammonemia, allowing further testing and evaluation of the method in practice.

*O11. Expanded Newborn Screening Combined with Second-Tier LC-MS/MS Methods*

Yosuke Shigematsu, Miori Yuasa, Ikue Hata and Go Tajima

University of Fukui, Department of Pediatrics, Fukui, Japan

**Introduction:** Newborn screening using tandem mass spectrometry (MS/MS) for a series of very rare inborn errors of metabolism causes high recall rate and many false positive cases. The second-tier tests by liquid-chromatography and tandem mass spectrometry (LC-MS/MS) using the first dried blood spots (DBS) can provide us diagnostic information about many treatable target disorders.

**Purpose:** Newly-developed second-tier tests by LC-MS/MS were evaluated for the target disorders of amino acid and organic acid metabolism.

**Materials and Methods:** Sciex API-4000 LC-MS/MS system and Shimadzu LC system equipped with a Intakt multi-mode column were used. Short-chain acylcarnitines, acylglycines, organic acids and amino acids in DBS were extracted from DBS (3 mm diameter) using methanol solution containing stable-isotope-labeled internal standards.

**Results:** In our MS/MS newborn screening, many positive cases with regard to such screening markers as C3-acylcarnitine (AC), C5-AC, C5-DC-AC, C5-OH-AC, and leucine/isoleucine were experienced. In our second-tier tests, increased concentrations of methylmalonic acid, 3-hydroxypropionic acid, and total homocysteine in the first DBS were useful to detect methylmalonic acidemia, propionic acidemia, disorders in cobalamin metabolism, and methionine cycle disorders. Those of 3-hydroxyglutaric acid, 3-hydroxy-3-methylglutaric acid were useful to detect glutaric acidemia type 1 and 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. Those of 3-methylcrotonylglycine, tiglylglycine, propionylglycine, isovalerylglycine were useful to detect multiple carboxylase deficiency, 3-methylcrotonyl-CoA carboxylase deficiency, 3-ketothiolase

deficiency, and isovaleric acidemia. Increased allo-isoleucine were specific to maple syrup urine disease. Recall rate was reduced significantly using our second-tier tests.

Conclusions: Our second-tier tests by LC-MS/MS were thought to be essential to perform the expanded newborn screening in view of cost effectiveness as well as accuracy.

#### *O12. Molecular Investigation in Chinese Patients with Short-Chain Acyl-Coa Dehydrogenase Deficiency*

Yanghui Zhang, Huiming Yan, Jing Liu, Zhengjun Jia and Hua Wang

Division of Medical Genetics, Maternal and Child Health Hospital of Hunan province, Changsha, China

**Introduction:** Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare autosomal recessive metabolic disorder of fatty acid beta-oxidation, and is associated with mutations of acyl-CoA dehydrogenase (ACADS) gene, leading to an abnormal level of butyrylcarnitine (C4-acylcarnitine, C4-C), butyryl glycine, butyrate and ethylmalonic acid (EMA). The incidence in Chinese population is proposed to be 1:84,000. Clinical features are identified to be two types. One is observed in infants with acute acidosis and muscle weakness, and the other is observed in middle-aged patients with chronic myopathy. Most individuals identified through newborn screening and affected relatives have been asymptomatic.

**Purpose:** We offer the patients of SCADD with etiologic diagnosis, genetic counseling and choices for therapeutic strategies according the mutation analysis.

**Materials and Methods:** Four patients of SCADD were identified by newborn screening and all of them were asymptomatic. The entire coding regions and intron—exon boundaries of ACADS were tested for mutations by Sanger sequencing.

**Results:** ACADS gene mutations were identified in all four patients of SCADD. Three of them had compound heterozygous mutations and one had homozygous mutation. Mutation c.1195C > T was novel.

**Conclusions:** Four patients were genetic diagnosed as SCADD by mutation analysis. The novel mutation c.1195C > T broaden the mutation spectrum of ACADS.

#### *O13 Enhancing Genetic Services in the Asia Pacific*

Carmencita Padilla MD

Newborn Screening Reference Center, National Institutes of Health, MAHPS, University of the Philippines Manila, Philippines

**Introduction:** The ultimate goal of every country is to integrate newborn screening (NBS) into the public health delivery system. As the newborn screening pilot evolves into a full program, managers realize the need for the development of basic infrastructure for genetic services. With the expansion of the newborn screening panel to include more genetically inherited conditions, there has been increasing demand for specialists to handle the management of patients diagnosed through the program.

**Purpose:** This session will present: (1) an overview on the existing genetic services in the countries in Asia; (2) formal and informal training programs in Clinical Genetics and Genetic Counseling; and (3) role of the Asia Pacific Society of Human Genetics in the provision of genetic services and training.

**Materials and Methods:** The geneticists and genetic counselors affiliated with the Asia Pacific Society of Human Genetics are the main sources of information.

**Results:** Review of country genetic programs reveals that development of services (in terms of clinical/human services, diagnostic/therapeutic, etc.) vary from country to country, depending on availability of genetic services that have been developed at the time newborn screening was introduced. Singapore, Malaysia, Thailand, Hong Kong, Australia, Philippines and India have trained clinical geneticists to render the clinical services. Universities and hospitals in China, Vietnam and Indonesia offer human genetic research and services. Indonesia, Philippines, Malaysia, Taiwan, and Australia offer a Master in Science in Genetic Counseling, to produce genetic counselors who will assist the

limited number of geneticists. The Asia Pacific Society for Human Genetics, a network of geneticists in the region explores creative ways of reaching out to countries with limited or no genetic services in the region.

**Conclusion:** There is a continuing demand for organized training in clinical genetics and genetic counseling to ensure that patients diagnosed through the NBS program receive proper management.

#### *O14. Iodine Deficiency and Iodine Excess-Two Sides of a Coin*

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**Introduction:** In India, population is likely to be prone to Iodine deficiency disorders due to deficiency of iodine in the soil of the subcontinent.

**Purpose:** This study was carried out to assess the current iodine status of our population and to ascertain if neonatal TSH is a surrogate marker for iodine repleteness of a population.

**Materials and Methods:** Neonatal TSH samples were collected as a part of Newborn screening project between 24 and 48 h. Urine samples were collected between day 2–7 postpartum from 400 and 144 mothers whose neonates had TSH value  $>5$  mIU/L and  $\leq 5$  mIU/L respectively and from 114 healthy pregnant mothers at term gestation.

**Results:** The iodised salt consumption in our population was 97%. The mean urinary iodine concentration in cases and controls were 240  $\mu\text{g/L}$  and 223  $\mu\text{g/L}$ . While iodine deficiency in both the groups were not significantly different, iodine excess was significantly higher in mothers whose neonates had TSH  $> 5$  mIU/L. The mean TSH was also significantly higher in iodine deficient and iodine excess group than iodine sufficient group. Mothers who underwent caesarean section had statistically high urinary iodine levels ( $p = 0.0001$ ). There was no significant correlation between neonatal TSH values and maternal urinary iodine levels. Among the pregnant mothers, 13.5% had iodine deficiency while 65.25% of the subjects had urine iodine levels more than adequate.

**Conclusion:** Iodine excess was present in the study population which could be attributed to the use of povidone iodine antiseptics in postpartum mothers and the improvement in Universal salt iodisation programme due to iodine excess in our pregnant mothers. Bearing in mind the recent studies on health hazards of iodine excess, use of known sources of iodine like povidone iodine antiseptics should be discouraged and regular monitoring of the iodine status through median urinary iodine levels and analysing the quality of iodised salt by national programmes are required.

#### *O15. Mass Urine Screening for Inherited Metabolic Disorders Using a Reliable and Inexpensive Thin Layer Chromatography Methodology*

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**Introduction-**The Provincial Neonatal Urine Screening Program for inherited metabolic disorders was instigated more than four decades ago in the Province of Quebec and the Nunavut region. It is supported financially by the Quebec Ministry of Health and Social Services.

**Purpose-**As part of a preventive genetic medicine program, the aim is to screen for disorders causing severe clinical problems which necessitate immediate therapeutic intervention, such as late-onset urea cycle disorders and organic acidurias and for those necessitating surveillance and follow-up in metabolic disorders and transport disorders.

**Materials and Methods-**Newborn urine samples are collected on filter paper (Whatman-GE 903) by parents at 21 days of age and sent to our centralized laboratory in Sherbrooke. Voluntary compliance is good averaging 90%. A multiplex thin-layer chromatography (TLC) methodology is performed, using

sequential application of four different reagents to detect aminoacidopathies and organic acidurias on the same plate.

**Results**—From 1973 to 2016, more than 3,200,000 babies have been screened. We detected 83 cases of methylmalonicaciduria, 8 methylcrotonylglycinurias, 4 oxoprolinurias, and 1 glutaric aciduria type I, while we confirmed 18 cases of argininosuccinic aciduria, 5 cases of citrullinemia type I (classic), 4 cases of citrullinemia type II, 4 cases of hyperargininemia, and 1 case of Triple H syndrome. The total incidence of disorders requiring early medical intervention is 1:25,000. We detected 193 cases of homozygous cystinuria and 1112 cases of heterozygous cystinuria, 16 cases of Fanconi syndrome, 71 cases of Hartnup disease and 73 cases of dicarboxylicaminoacidurias with a total incidence of cases requiring counselling and surveillance at 1:2200. The cost is 4.50\$CAD/newborn.

**Conclusions**—This technical approach is simple, reproducible, rapid and inexpensive, allowing the analysis of 500 samples daily by a single technician. The use of filter paper facilitates the urine collection by parents, shipping by mail and storage of samples.

#### *O16. National Newborn Blood Spot Screening*

Veronica Wiley, Craig White, Karla Lister and Faye Bowman

The Children's Hospital at Westmead, NSW Newborn Screening Programme, WESTMEAD, Australia

Newborn screening programs provide coverage for a defined geographical region covering all or part of a state, province or prefecture of a country. There are a few, like that in New Zealand, where all samples for the country go to one centre. In most countries, there needs to be co-ordination of the service offered.

In Australia, newborn blood spot screening is offered to all babies born (approximately 330,000 births per year) by one of 5 publicly funded state based programs. Whilst guidelines for newborn screening practice are offered by professional societies, it is up to each state program to decide what is included and therefore the screening pathway and disorders screened for a baby depends on where it is born.

In order to provide national consistency and set a clear pathway for deciding what should be included in the future, the Standing Committee on Screening (SCOS) formed a Newborn Bloodspot Screening Working Group (NBSWG). This included representatives from state and federal governments, professionals from screening for program and laboratory aspects, other health professionals, a bioethicist as well as a consumer representative.

The NBSWG has used a consultative approach, contacting health professionals and consumers before drafting a framework. The policy, which includes program overview; program implementation; quality and safety; monitoring, evaluation and review; and decision-making framework has been submitted and the disorder decision making framework is being stringently evaluated. It is anticipated that federal government will endorse the submission and a national policy will be applied.

In conclusion, a policy built on the strengths and successes of the last 50 years of screening in Australia will provide clear policy guidance, support consistency, with increased transparency as well as enable structured assessment of disorders.

#### *O17. Are We Doin' It Good Boss?—Quality in Newborn Screening*

Rosie Junek and Veronica Wiley

The Children's Hospital at Westmead, NSW Newborn Screening Programme, Wentworthville, Australia

**Introduction:** There are many things to consider when assessing what is "quality newborn screening". A newborn screening program encompasses pre-testing; sample testing and patient follow up. Quality improvement involves assessment of all aspects of our programme.

**Purpose:** Our aim is to maintain and improve a high quality screening programme.

**Materials and Methods:** The NSW Newborn Screening Programme is under constant review to improve the quality of what we do. We observe aspects that are both internal and external to the laboratory. As a programme we deal with what happens before the sample is collected, collection of the sample, testing of the sample, delivering results and follow-up till diagnosis or exclusion of a disorder. Methods used are diverse and can be through database information, questionnaires, community awareness and observation. Each aspect of the process is reviewed by members of our team to look for ways to improve the quality of our programme.

The National Association of Testing Authorities (NATA) accreditation processes look at quality of the scientific portion of the program.

**Results:** Currently, we use Human Genetics Society of Australasia key performance indicators to assess some pre and post analytical aspects of screening quality.

Indicator	Target	Our Result 2007	Our Result 2014	Our Result 2016
Percentage of specimens in transit for 4 days or less	95%	76%	87%	85%
Percentage of unsuitable sample	Less than 0.5%	0.59%	0.58%	0.54%
Median age at specimen collection	95% at 48–72 h	Day 3	Day 2	Day 2

For all laboratory performance, the NSW Newborn Screening Programme is accredited to ISO15189 by NATA.

**Conclusions:** Quality is not static and needs to be evaluated frequently. It is important to schedule review and, if appropriate, update any and all aspects of newborn screening, not just scientific methods.

#### 4. Poster Presentations

##### *P1. Assessing Performances and Usability of the NS2400 Automated Platform from Labsystems Diagnostics*

Lotta Virtanen

Labsystems Diagnostics Oy, Research & Development, Vantaa, Finland

**Introduction:** Newborn Screening in most countries is highly centralized with daily sample loads of 300 to over 2000 in a few cases. Therefore, totally automated systems are highly desirable. The main advantage of these systems is less hands-on-time for the laboratory personnel, better traceability of the working steps, and less danger of mistakes and false screening results. The NS2400 from Labsystems Diagnostics combines total automation by replacing exactly the technician processed without altering the product design. The same kit reagents can be used as manually and/or be integrated in the NS2400 platform ensuring continuity of measurement in case of downtime. The automate's modules can also be used as standalone providing further flexibility of use.

**Materials and Methods:** We tested the system thoroughly with all so far available tests (TSH, 17-OHP, IRT, Biotinidase, total Galactose, G-6-PDH, and PKU) and comparison with routine tests are reported.

**Results:** G-6-PDH gave equivalent results with our in-house test, and >80 samples from external QC scheme over 2–12 years gave correct values and correct assignments. Tests for IRT and biotinidase showed less variation and resulted in smaller numbers of false positives compared to the routine test. TSH showed very good correlation with a mean difference of 0.77 mIU/L.

**Conclusion:** The automateted assay platform NS2400 from Labsystems proofed to be a robust, reliable, and flexible instrument for newborn screening. With our retrospective data we could demonstrate a much lower recall rate for biotinidase and IRT, compared to the routine kits.



## P2. Some Results of First Newborn Hearing Screening in Mongolia

Saruul Chuluunbaatar and Delgermaa Bataakhushg

Pediatric Clinic, MCHRC, Department of Otorhinopharyngology, Ulaanbaatar, Mongolia

**Purpose:** Our study aimed to detect hearing loss and deafness among infants as early as possible with the automated auditory brainstem response.

**Materials and methodology:** We started the screening from December 2012 and by the second quarter of 2016 we were able to involve 9379 newborns in total. We tested automated auditory brainstem response of infants in their 1–3 days of birth using Maico MB11 Beraphone machine (Germany) according to the guidance from Joint Committee on Hearing Screening. We assessed the test result of “pass” as normal hearing, “refer” as to rescreen and tested the infants again after 1 month. For the second occurrence of “refer” result we dispatched the patients for the higher level specialized audiologic tests. We did ABR test for infants when they reached 3 months old.

**Results:** Our study was the first in Mongolia and 8023 of 9379 studied newborns tested with result of “pass” and 1356 with “refer”. In the rescreen test, 447 of 546 infants resulted with “pass” and 99 with “refer”. For those 99 infants we did specialized audiologic tests in their age of 3, 6, 9 months old, and we revealed 6 children (12 ears) having bilateral profound hearing loss, 3 children (6 ears) having bilateral mixed profound hearing loss. The first “refer” rate was 14.4%, the second was 4.5%. The follow rate was 40.2%. We are preparing 0–3 years old children with bilateral profound hearing loss for the follow-up CI surgery.

**Conclusion:** In Mongolia, the data of infant hearing difficulties and prevalence of deafness is lacking. Although newborn hearing screening program, approved in 2014, brought opportunities to conduct universal neonatal hearing screening, is available only at NCMCH. Our study’s preliminary result shows similar pattern (1.1–1.3 ear deafness in every 1000 birth. Lenarz et al., 2008) of 9 cases of bilateral profound hearing loss out of 9379 newborns.

## P3. Comparison of Use in Routine Practice of Neonatal Screening for Congenital Hypothyroidism—Automatic Methods of Analysis: (Autodelfia) and Manual Method (Manual Delfia)

Vyacheslav Mitkin

Science Psychiatric Children Center, Center of Neonatal Screening, Moscow, Russia

The aim of the study is to compare the qualitative characteristics of neonatal screening using automated systems for sample preparation and analysis (Autodelfia) and manual method of sample preparation using the analyzer Victor in a routine screening assay. Were compared in parallel (counts of fluorescence) in the standards and controls for kit Cat. N B032-312 Autodelfia NeoTSH ( $n = 34$ ):

	STANDARD N1	STANDARD N2	STANDARD N3	STANDARD N4	CONTROL
	(0.9 $\mu$ U/mL)	(9.5 $\mu$ U/mL)	(25.6 $\mu$ U/mL)	(111.0 $\mu$ U/mL)	(45 $\mu$ U/mL)
AUTO	1449 $\div$ 110	8712 $\div$ 364	22243 $\div$ 789	81370 $\div$ 3347	12491 $\div$ 1095
(counts)	(7.6%)	(4.2%)	(3.6%)	(4.2%)	(8.8%)
MANUAL	1206 $\div$ 226	7295 $\div$ 896	17012 $\div$ 2549	66110 $\div$ 10168	10933 $\div$ 1849
(counts)	(18.8%)	(12.3%)	(15%)	(15.4%)	(16.9%)

We have found when using manually method the decrease in the absolute account for fluorescence of all measured standards and controls on the average on 15–20%, in addition, the standard deviation is in 2–3 times higher in manual method than in auto. If we compare the calculated concentrations in the controls, we found also very considerable variation in standard deviation:  $14.4 \pm 1.4 \mu$ U/mL for auto in compare with  $15.5 \pm 3.5 \mu$ U/mL in case of manual method.

These data suggest the importance of standardization and unification of procedures of sample preparation in neonatal screening and indicates for use a preferably of automatic method of sample

preparation the type of Autodelfia. These data indicate not only the importance the human factor but also the need to control of all steps of sample preparation (washing, shaking, dispensing etc.) in manual method. This is especially important in the case of using screening technologies for filter the blanks in prenatal screening where the quality of the analysis of biochemical markers directly affects the effectiveness of prenatal screening in general.

#### *P4. Analysis of Neonatal Congenital Hypothyroidism Screening from 2007 to 2016 in Zhejiang Province*

Xiaocha Xu, Dingwen Wu and Huaqing Mao

Children's Hospital, Zhejiang University School of Medicine, Central laboratory of Neonatal Screening of Inherited Metabolic Diseases, Hangzhou, China

**Objective:** To analyse and determine neonatal Screening coverage and incidence of congenital hypothyroidism (CH) from 2007 to 2016 in Zhejiang Province.

**Methods:** The screening for CH are based on the measurement of TSH in dried blood spots using time-resolved fluoroimmunoassay, diagnosis using chemiluminescence by detection of serum free T3 (FT3), FreeT4 (FT4) and TSH.

**Results:** In 2007–2016, there were 6,335,190 newborns were screened for CH from 6,444,265 live births, the total screening coverage was 98.31%, and the coverage were in more than 99% per year recently three years. CH and subclinical hypothyroidism were detected in 4220 newborns and 1794 newborns, respectively, the total incidence of CH and subclinical hypothyroidism was 1/1056 (5994/6,335,170), and annual incidence increased year after year even to 1/731 until 2016.

**Conclusion:** At present the neonatal screening coverage is above 99% in Zhejiang province, but the incidence of CH increase year after year. It should be continued to proceed with the neonatal screening programs, and improve the neonatal screening coverage, effectively reduce the happening of the defective child.

#### *P5. To Study the Screening Tsh Cut-Off Value, the Clinical Features and Outcome of Congenital Hypothyroidism: A-15-Year Experience*

Rulai Yang, Zhengyan Zhao, Tong Fan, Fang Hong, Xuelian Zhou, Dingwen Wu and Huaqing Mao

Children's Hospital of Zhejiang University School of medicine, Department of genetic and metabolism, Hangzhou, China

**Objective:** To study the screening TSH cut-off value of congenital hypothyroidism (CH), the different clinical features of permanent congenital hypothyroidism (PCH), transient congenital hypothyroidism (TCH), preterm CH and low-birth-weight newborn CH. The prognosis influencing factors of PCH and TCH were also analysed.

**Method:** Clinical data of newborn CH, who were diagnosed and followed-up in the neonatal screening centre from August 1999 to April 2013. A total of 2789 cases of CH were enrolled in this research. According that if the cases stop L-Thyroxine at their 2 or 3 years old, they were assigned into two groups: PCH group ( $n = 682$ ) and TCH group ( $n = 621$ ). The clinical features, prognosis, prognosis relevant factors were studied comparatively to PCH and TCH. And the clinical features of preterm CH and low-birth-weight newborn CH were also described.

**Result:** The neonatal screening incidence of CH was 1/1886 in this study, the screening TSH level  $\geq 10$  mIU/L accounted for 97.5% cases. The 682 cases of PCH and 621 cases of TCH were diagnosed. The radio of male/female and mean screening TSH value were higher of PCH group than of TCH group (4.44:1 vs. 1.14:1 and  $96.4 \pm 82.3$  mIU/L vs.  $46.8 \pm 46.0$  mIU/L, respectively, all of the  $p < 0.01$ ). While the infant's age of serum FT4 values recovered, and the normal percentage of stature, bone age, DQ assessment were lower of PCH group than of TCH group ( $57.8 \pm 16.4$ d vs  $68.2 \pm 75.7$ d, 87.9% vs. 99.0%, 92.9% vs. 96.7% and 95.1% vs. 98.4%, respectively, all of the  $p < 0.01$ ). There were 117 (PCH 22.2%) cases of preterm CH and 111 cases (PCH34.2%) of low-birth weight newborn CH being found.

There were 56 cases (8.2%) in PCH group reusing L-Thyroxine during followed-up. The complicated incidence in 2789 cases of CH was 2.1%.

**Conclusion** The recommended screening TSH cut-off value of CH is >9 mIU/L. The outcome of TCH was better than of PCH. The high screening TSH level implies PCH. Preterm CH and low-birth-weight newborn CH need to be treated standard. CH cases need long-term following-up and systemic examining.

*P6. Experience of the Quantitative G6PD Assay on the Automated GSP®; System in a Routine Newborn Screening Laboratory, Verona, Italy*

Hanna Polari, Marta Camilot, Francesca Teofoli, Liisa Meriö, Teemu Korpimäki, Pauliina Mäkinen, Sari Airene and Harri Hakala

PerkinElmer, Neonatal Screening, Turku, Finland

**Introduction:** Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common disorder in Italy, the prevalence being as high as 11% in particular regions. G6PD is part of the disease panel the centre of newborn screening laboratory of Verona, Italy, screen for. Due to X-inactivation, heterozygous females can be partially deficient but the severity varies, which makes female G6PD screening challenging. However, since G6PD deficient newborns are specifically susceptible, it would be of importance to find also the severe heterozygous females in a timely manner.

**Purpose:** The objective was to evaluate the screening performance of an automated GSP®; Neonatal G6PD assay (PerkinElmer Inc.) in screening newborns for G6PD deficiency, both in boys and girls. The capability of the GSP®; Neonatal G6PD assay to detect the female carriers was also evaluated.

**Material and Methods:** G6PD activity was measured in a total of 61,963 newborn dried blood spot specimens (32,202 M and 29,761 F), taken from heel prick at 36–48 h of life and measured using GSP®; Neonatal G6PD kit; the descriptive statistics including median, range and lower percentile cut-off were calculated. The status of deficiency or carrier was confirmed by whole blood quantitative determination.

**Results:** Two-sample Wilcoxon rank-sum-; (Mann-Whitney) test was used to detect significant difference between female and male G6PD activity on blood spot. The median G6PD activity of routine neonatal samples was 69.9 U/dL blood (range: 0 to 252 U/dL) and the 3rd lower percentile corresponding to a value of 26 U/dL blood was set as the cut-off, both on males and females. The algorithm for the recall allows the detection of female carries along with complete deficiencies, both in males and females.

**Conclusions:** The results suggest that the GSP®; Neonatal G6PD kit is suitable for routine high-throughput newborn screening, with the potential for finding female carriers.

*P7. Evaluation of a New Non-Derivatized Mass Spectrometry Kit for Newborn Screening*

Ping-chun Wang, Yu-shan Tseng, Shiao-fang Wang, Li-hsin Chen, Shin-chuan Tseng, Pin-wen Chen and Yin-hsiu Chien

National Taiwan University Hospital, Medical Genetics, Taipei, Taiwan

**Introduction:** The PerkinElmer®; NeoBase™ 2 newborn screening kit uses a non-derivatized MSMS method to measure blood levels of amino acids, free carnitines, acylcarnitines, lysophospholipids and purine nucleosides in newborns. The abnormal levels of these markers may be associated with disorders of inborn error of metabolism.

**Purpose:** Current method of MS/MS in our laboratory is time-consuming and utilizing hazardous reagents such HCl-butanol. We evaluated a new, commercial kit with a significantly less harmful derivative and saved sample preparation time.

**Materials and Methods:** The precision and clinical evaluation were performed by two levels of quality controls, proficiency testing samples from Centers for Disease Control and Prevention (CDC, Atlanta, USA) and dried-blood spots of 7545 de-identified newborns.

**Results:** The precision was shown to be acceptable with approx.  $CV \leq 14\%$  and cutoff values were determined with normal neonates and confirmed retrospective specimens. Sample preparation time can be reduced significantly to 60 minutes from 120 minutes. To date, we have screened 28,644 neonates and identified 7 cases of inborn errors of metabolism (IEM); 1 for 3-MCC deficiency, 2 for maternal primary carnitine deficiency, 1 for 2MBCDD, 1 for MMA, 1 for PKU and 1 for citrullinemia II.

**Conclusions:** The non-derivatized MSMS assay was demonstrated to be accurate in the detection of newborns with IEM and without the risk of the exposure to highly toxic reagents and requirement of additional equipment for toxic fume evacuation. This new kit could incorporate new markers and enhanced functionality. Screening of neonates with IEM before symptomatic onset, they could receive early treatment, medications and nutrients supplements with support and assistance from National Health Insurance Program or Administration of Health Promotion in Taiwan. For families and society, tandem mass spectrometry screening was shown to allow normal growth and development of affected neonates and reduction of financial costs.

#### *P8. Tandem Mass Newborn Screening in Taiwan—Report from One Newborn Screening Center*

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**Introduction:** Newborn screening (NBS) using tandem mass spectrometry (MS/MS) started in Taiwan in 2000. The outcomes of MS/MS screening had shown that it is a reliable method for the early diagnosis of many metabolic disorders and it might also significantly improve the quality of life of affected newborns. Therefore a nationwide expanded NBS accompanied with a pilot study were commenced from 1 July 2006. Between expanded NBS and pilot study, there are more than 22 diseases detected by MS/MS in Taiwan.

**Purpose:** Tandem mass NBS is including disorders of amino acid, organic acid metabolism and fatty acid oxidation. We analysed the frequency of diseases to propose report for policy makers in planning of NBS.

**Materials and Methods:** Between 2007 and 2014, 604,014 neonates were screened by MS/MS. Dried blood spot (DBS) samples were collected after 48 h at birth with parental consent.

**Results:** In total, 231 cases of inborn errors of metabolism were detected. The overall incidence was one per 2614 births. The most common disease found was 3-methylcrotonyl CoA carboxylase deficiency. The second most common disease was primary carnitine deficiency. Among these affected newborns, study had revealed a relatively high incidence of citrullinemia.

**Conclusions:** MS/MS screening resolved the unique spectrum of metabolic disorders in Taiwan. In addition to the nationwide expanded NBS, some disorders in pilot study should be considered for implementation of NBS. In the early diagnosis of severe and treatable inborn errors of metabolism such as organic acidurias and urea cycle disorders, MS/MS screening was shown to be economically valid with significant reduction in critical care and chronic medical care expenses.

#### *P9. Improved Tandem Mass Spectrometry Newborn Screening: New Neobasetm2 Non-Derivatized Msms Kit Assay Features and Performance as Compared to the Current Neobasetm Assay*

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PerkinElmer, Newborn Screening, Turku, Finland

**Background and objectives:** PerkinElmer NeoBase™2 Non-derivatized MSMS Kit (NB2) is a novel, next generation tandem mass spectrometry (MSMS) kit for improved newborn screening. As compared to the current world-widely used NeoBase™ Non-derivatized MSMS Kit (NB), NB2 allows shorter plate preparation and total assay times, and provides further expanded screening panel with additional novel markers including glutamine, glutamic acid, argininosuccinic acid, five new longer chain acylcarnitines, four lysophospholipids and two purine nucleosides adenosine and deoxyadenosine. The objectives of this study were to demonstrate the benefits of NB2 assay, and provide typical analytical performance comparison between NB and NB2.

**Methodology:** Dried blood spot (DBS) specimens were prepared from human whole blood enriched artificially with multiple control analytes included in both kits. All samples were tested with both assay protocols to study their between method correlation. The DBS disks were punched and extracted on 96-well microplates with the extraction solution including specific stable isotope-labelled internal standards (IS) for each marker and/or marker group. The prepared test plates were analysed with both MSMS assays on Waters TQD MSMS system using direct-infusion positive electrospray ionization (ESI+) method and multiple reaction monitoring (MRM) mode.

**Results and conclusions:** Study results suggested that the compared assays showed relatively good overall correlation with majority of analytes including multiple amino acids, acylcarnitines and succinylacetone. However, due to several procedural improvements implemented in the NB2 assay (e.g., 75 min shorter plate preparation time, optimized labelling of alanine and valine ISs, and improved MSMS flow solvent composition), some expected systematic differences with several analyte result levels were found. This means that all screening laboratories aiming to update to NB2 should run both assays in parallel for a transition period prior to assay change and update their current reference ranges accordingly.

#### *P10. A Novel LC-MS Based Technique to Detect Msud and Methyl Malonic Aciduria from Urine*

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**Introduction:** Inherited metabolism disorders include a variety of disorders which are individually rare but frequently observed, affecting the pediatric population causing mortality or morbidity. Newborn screening for IEM imparts an opportunity for pediatric patients at high risk to identify these conditions and treat them if possible. LC-MS based techniques even though sensitive and specific are very scarce until recently because of the unavailability of the interface such as ESI technique to couple LC-MS.

**Purpose:** To develop and validate a LC-MS based analytical method for the simultaneous determination of biomarkers responsible for MSUD and MMA such as methylmalonic acid, 3-methyl-2-oxovaleric acid and 2-hydroxy-3-methylbutyric acid.

**Materials and methods:** The analytical method was developed for screening biomarkers responsible for maple syrup urine disease and methyl malonic aciduria on a Thermo LC-MS LTQ ion trap system and have used HILIC column for the efficient separation of polar organic acids.

**Results:** The heated ESI source conditions and Ion optic conditions were optimized for the MS/MS after achieving a good response in the tuning study using a mobile phase combination of formic acid in acetonitrile: formate buffer. Chromatographic conditions were optimized using the HILIC column (250 mm × 4.6 ID, 3 micron). Tropic acid was found to be a suitable internal standard. The retention times were 2.20, 3.00, 3.40, and 3.50 min respectively for 3-methyl-2-oxovaleric, MMA, 2-hydroxy-3-methoxybutyric acid and Tropic acid respectively. The developed method was then validated for its suitability for routine use as per the USFDA guidelines. The stability study results for the biomarkers were all within the acceptable limits. The developed and validated method was later successfully applied for analysis of pediatric samples for MSUD and methyl malonic aciduria.

**Conclusion:** A simple, rapid and sensitive LC-MS based analytical method was developed and validated to detect MSUD and methyl malonic aciduria from urine.

*P11. Mutational Analysis of SLC22A5 Gene in 21 Patients with Primary Carnitine Deficiency*

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**Objective:** Primary carnitine deficiency (PCD) is an autosomal recessive disorder that impairs fatty acid oxidation, caused by homozygous or compound heterozygous mutation in the SLC22A5 gene on chromosome 5q31. It has a frequency of ranging from 1:40,000 to 1:120,000 newborns in different parts of the world. The clinical manifestations of PCD can vary widely with respect to age of onset, organ involvement, and severity of symptoms. The most common presentations are in infancy and early childhood with either metabolic decompensation or cardiac and myopathic manifestations, respectively. The major clinical diagnose of PCD is based on the level of creatine kinase. The final diagnosis of PCD can be confirmed by SLC22A5 gene analysis. Through the tandem mass spectrum analysis, 17 newborns were considered to be PCD and another 4 were considered to be carrier of PCD. Analysis of SLC22A5 was followed up to ascertain the genetic background.

**Results:** Total detection rate of gene mutation is 100% in the 17 patients and 4 possible carriers of PCD, c.760C > T (p.R254X) was the most frequently seen mutation. And in this study, we detected 4 mutants (c.495C > A.c.1298T > C.c.288delG and c.774\_775insTCG) which have not been reported.

**Conclusion:** This study confirmed the diagnosis of 17 patients and 4 carriers with PCD on the gene level, 4 novel mutations were detected, which expanded the mutational spectrum of the SLC22A5 gene.

*P12. Novel Single Nucleotide Polymorphism of Phenylalanine Hydroxylase Gene Causes False Diagnosis of Homozygous Mutation*

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**Introduction:** Phenylketonuria (PKU) is the most common autosomal recessive disorder worldwide, with an overall incidence in China of 1 in 11,144.

**Purpose:** To perform genetic diagnosis for a patient with phenylketonuria (PKU) and his family.

**Materials and Methods:** DNA samples from the proband and family members were sequenced by polymerase chain reaction, including all 13 coding exons and flanking regions of the phenylalanine hydroxylase gene (PAH).

**Results:** Sequencing showed compound heterozygous mutations in the PAH gene in the proband, namely c.721C > T and c.1068C > A, with paternal and maternal origins, respectively. However, a homozygous c.1068C > A mutation detected in the proband's mother using common primers subsequently turned out to be heterozygous when examined using new primers and re-sequenced. A novel single nucleotide polymorphism (SNP) c.1066-193G > C was found in the common forward-primer-binding area on the normal allele. The frequency of this novel polymorphism was 0.027, based on 300 chromosomes from the general population.

**Conclusions:** Our results suggest that allele dropout may occur when SNPs are located in the primer-target sequence, and we therefore propose that a new primer should be used for mutational analysis of exon 11 of PAH to avoid misdiagnosis.

*P13. Carnitine-Acylcarnitine Translocase Deficiency: Case Report*

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Carnitine-acylcarnitine translocase deficiency (CACTD) is a life-threatening, recessively inherited disorder of lipid  $\beta$ -oxidation which manifests with hypoketotic hypoglycemia, cardiomyopathy, liver failure, and muscle weakness. Here we first report two cases of CACTD from mainland China. The first proband had homozygous mutation of c.199-10T > G in SLC25A20 gene, suddenly died of cardiac arrest on the 3rd. The second case presented with circulatory disturbance was quickly suspected CACTD or carnitine palmitoyl transferase II (CPT-II) deficiency by tandem-mass-spectrometry (MS/MS). She was improved by high glucose infusion, intravenous arginine, and circulatory support, but cannot remove ventilator for a long time. The same mutation c.199-10T > G and a novel mutation c.1A > G in SLC25A20 was identified. We present the clinical and biochemical features of the probands and eleven other previously reported cases with c.199-10T > G mutation, to better understand the genotype/phenotype correlation of this disease, the poor prognosis of this form, and urge pediatricians working with a Chinese population to look out for CACTD.

#### *P14. Molecular Characterisation of Hyperphenylalaninemia in Korea*

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Phenylketonuria (PKU) is an inherited disorder characterized by increased level of phenylalanine in the blood. PKU is frequently caused by functional deficiency of phenylalanine hydroxylase (PAH), an enzyme that converts phenylalanine to other compounds in the body. More than 900 mutations have been identified in PAH, and recorded in the locus-specific database (LSD) known as PAHvdb. The occurrence of PKU varies among ethnic and geographic regions, reaching approximately 1 in 15,000 newborns. Here we report a spectrum of PAH mutations compiled from 128 patients from Korea. The all 13 exons and adjacent intronic regions of the PAH gene were determined by Direct sequencing. We identified 50 different mutations, of which 5 are not reported before. Several mutations reoccurred with high frequency including R243Q (18%), Y204S (12%), IVS4-1G > A (7.8%), Y356XA (7.8%), R241C (7.8%), A259T (6.1%), T278L (6.5%). Although some common characteristics of allele frequency and distribution were identified among oriental populations, several distinctive characteristics were revealed in Korean patients. Although the R413P allele is the most prevalent form (30.5%) in Japanese, we detected it in only six alleles from 256 independent alleles (2.3%). The A259T allele, which has not yet been found in oriental populations, was frequently found in this study. We also observed that tetrahydrobiopterin (BH4) responsiveness was associated with specific genotypes (R53H, R241C, R408Q and T278L), suggesting there are some correlations between phenotype and genotype. 6-pyruvoyltetrahydropterin synthase (PTPS) deficiency was associated with genotypes (c.272A > G, c.347A > G, c.259C > T, c.616A > G, c.155A > G).

#### *P15. Six-Plex Fia-MS/MS Assay to Measure ABG, ASM, GAA, GALC, GLA and Idua Enzyme Activities in Neonatal Dried Blood Spots*

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A new multiplex flow injection analysis tandem mass spectrometer (FIA-MS/MS) method is described that simultaneously measures the activities of the enzymes ABG, ASM, GAA, GALC, GLA and IDUA, using a single 3.2 mm punch from a dried blood spot (DBS). Modifications to the substrates and internal standards used by other methods combined with a fully optimized buffer system and simplified procedure allow for more efficient measurements and the ability to obtain 576 activity results from one 96-well plate.

The streamlined method comprises nine steps from the initial DBS punch to the final analysis by FIA-MS/MS. After an overnight incubation, the sample processing can be completed within 30 minutes per 96-well plate, using a liquid handler or manually as one desires. The sample to sample analysis time on the mass spectrometer is approximately two minutes using isocratic flow for sample delivery.

The six-plex method was tested with DBS from presumed healthy subjects and with samples having confirmed low activities, as well as with CDC DBS controls. The data showed a robust difference between the enzyme activities from the two sample types with acceptable signal-to-noise and precision.

*P16. An Automated DBS DNA Extraction Method for a Five-Plex QPCR Assay That Determines Copy Numbers of SMN1, SMN2, TREC, KREC, and Rpp30*

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A five-plex real-time PCR assay was developed to measure copy numbers of five different loci using DNA extracted from a single 3.2 mm punch of a dried blood spot (DBS). A simple buffer DNA extraction method was developed for a Janus<sup>®</sup>; liquid handler that can process 384 DBS punches in four 96-well plates in just over one hour. The PCR assay identifies the absence of exon 7 in the SMN1 gene while simultaneously evaluating the copy number of the SMN2 gene. This is achieved by using specific Locked Nucleic Acid (LNA<sup>®</sup>) Taqman<sup>®</sup>; probes for both the SMN1 and SMN2 genes. The LNA<sup>®</sup>; Taqman<sup>®</sup>; probes for SMN1 and SMN2 differ in sequence by a single nucleotide polymorphism (SNP). Elevated annealing temperatures and different fluorescent labels on the LNA<sup>®</sup>; Taqman<sup>®</sup>; probes permitted the simultaneous interrogation of both loci. To further demonstrate the capability of a multiplex assay, PCR primers and standard dual labelled Taqman<sup>®</sup>; probes for T-cell receptor excision circles (TREC) and for K-deleting recombination excision circles (KREC) were included. Additionally, the amplification of a reference gene, RPP30, was included in the assay as a quality/quantity indicator of DNA isolated from the DBS.

The five-plex real-time PCR assay performance was demonstrated on ~1000 DNA samples isolated from 3.2 mm punches of de-identified putative normal newborn DBS. The DNA was extracted from the DBS samples with a simple isolation buffer on a Janus<sup>®</sup>; liquid handler instrument. Finally, the five-plex assay performance was demonstrated on contrived DBS control samples in which linear synthetic DNA targets or cells were spiked into leukocyte depleted blood products that were spotted on Ahlstrom<sup>®</sup>; 226 filter paper. The results from this study with a five-plex real-time PCR assay demonstrate the potential of future molecular DBS assays.

*P17. A Pilot Study for Newborn Screening of X-Linked Adrenoleukodystrophy*

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**Introduction:** X-linked adrenoleukodystrophy (X-ALD) is a rare inherited metabolic disorder and is caused by mutations in the ABCD1 gene encoding a peroxisomal ABC transporter ALDP. X-ALD can show a variety of phenotypic manifestations from severe childhood cerebral ALD to adult-onset adrenomyeloneuropathy. Recent studies have suggested that C26:0-lysophosphatidylcholine (C26:0-LPC) can be a sensitive biomarker for X-ALD before the onset of manifestations.

**Purpose:** Using high-throughput screening by C26:0-LPC in dried-blood spots (DBS) by tandem mass spectrometry were reported by different laboratories in recently years. Consequently newborn screening of X-ALD has been proposed to allow timely diagnosis, monitor and treat at an early disease stage for this severe disorder. To investigate the rates for incidence, provide genetic counseling and treatment option, we have conducted a pilot newborn screening program for X-ALD in Taiwan.



**Materials and Methods:** C26:0-LPC levels were measured in DBS for routine newborn screening for inborn errors of metabolism. First tier screening is performed using MS/MS and 2nd tier screening is accomplished by LC-MS/MS with a first-tier result of  $\geq 0.3 \mu\text{M}$ .

The recall DBS were obtained with a 2nd tier result of  $\geq 0.4 \mu\text{M}$ . If positive of a recall DBS, further confirmation tests including physical examination and other methodology for ALD confirmation will be provided. To monitor the test performance, proficiency testing from Centers for Disease Control and Prevention (CDC, Atlanta, GA, USA) was enrolled.

**Results:** From 1 November 2016 to 30 April 2017, 32,074 newborns have been screened for X-ALD. One newborn was recalled and had normal result. In DBS from 32,074 newborns C26:0-LPC were  $0.181 \pm 0.051 \mu\text{M}$ , while in one retrieved newborn DBS of X-ALD patient C26:0-LPC was  $1.056 \mu\text{M}$ .

**Conclusions:** Preliminary results showed that C26:0-LPC can be reliably detected in DBS. Levels of C26:0-LPC showed significant elevation in DBS of X-ALD patient compared to controls.

#### *P18. 34 Years Screening for Duchenne Muscular Dystrophy in Germany, 1977–2011*

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This is a personal report about screening of 528,410 mostly 4–6 weeks old boys in Germany between 1977 and 2011 for high activities of creatine kinase (CK) to detect boys with Duchenne and Becker muscular dystrophy (DMD, BMD). During these 34 years of infant screening, 147 boys with confirmed, probable and possible Duchenne dystrophy (1:3600) and 33 with confirmed, probable and possible Becker dystrophy (1:16,000) were found. Personal and continuous information for families and pediatricians about DMD research accompanied the screening. CK screening programs like this one and therapies for pre-symptomatic DMD boys to slow down the progression of the disease—like in BMD—; are discussed for keeping the Duchenne symptoms of boys with dystrophin mutations as acceptable as possible. However, new dystrophin mutations will continue to occur, thus screening and early therapy will always be needed. A newly detected benign, dominant inherited blood anomaly with CK-BB isoenzyme in the particulate portion of the blood was found in 138 boys (1:3800).

#### *P19. Analytical Performance Characteristics of an Automated Creatine Kinase Muscle Isozyme Immunoassay*

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**Introduction:** Creatine kinase (CK) is an enzyme that catalyses the ATP-driven phosphorylation of creatine to phosphocreatine. CK has various isozymes, of which CK-MM is the one predominantly present in skeletal muscle cells. Any severe damage to skeletal muscle cells leads to the release of CK-MM into the blood. Thus the presence of CK-MM in blood is a biomarker for skeletal muscle damage causing disorders, such as Duchenne muscular dystrophy (DMD), which is a progressive and eventually lethal neuromuscular disorder with a world-wide incidence of 1:5000 live male births.

**Objective:** Our objective was to determine analytical performance characteristics of an automated immunoassay for measuring CK-MM from dried blood spots (DBS), which is currently under development for the GSP<sup>®</sup>; system from PerkinElmer.

**Methods:** DBS samples were prepared from adult human whole blood or washed red blood cell suspension and the CK-MM concentration was adjusted by spiking with purified human CK-MM. The precision, detection capability, linear range, high dose hook effect and analytical specificity of the GSP<sup>®</sup>; CK-MM method were determined, following the guidelines of the Clinical and Laboratory Standards Institute. The performance of the GSP<sup>®</sup>; CK-MM method was compared to an enzymatic CK activity assay (the Orphanos method) using 20 archived de-identified neonatal DBS samples from DMD patients and approximately 700 time-matched unaffected specimens.

**Results and Conclusions:** The results are expected to support a measuring range spanning at least from 30 to 6000 ng/mL in whole blood, which would be suitable for measuring most neonatal DBS samples. No major interference effects by relevant endogenous or exogenous compounds are expected. The results of the GSP®; CK-MM method are expected to correlate with the level of CK activity in the DBS samples.

*P20. The Biochemical and Genetic Analysis of Biotinidase Deficiency in the Population of Taiwan*

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**Background:** Biotinidase deficiency (BD) is an autosomal recessively inherited disorder of biotin recycling that is associated with neurologic and cutaneous presentations if untreated. Newborn screening for BD can benefit patients especially to those with profound BD (below 10% of mean normal activity). However, the incidence of BD in Taiwan is unknown.

**Method:** Dried blood samples were taken from newborns at the age of 2–3 days as other newborn screening items. The biotin activity in dry blood spots was determined using a quantitative fluorescence assay. Newborns who were deficient in biotinidase activity (AG (p.V417E)). However, all of them were classified as partial BD, and they have Biotin supplement only when illness. The incidence of BD in this population was 1 in 31,451 (95% confidence interval, 1 in 10,697 to 92,480).

**Conclusion:** The incidence of BD is 1 in 31,451, but all belong to partial BD only. The true incidence of profound BD is less than 1 in 94,354 newborns.

*P21. Analysis of Physical and Chemical Factors on the Quality of Dried Blood Spots during Preparation Based on 4 Neonatal Screening Assays*

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**Objective:** To investigate the effect of physical and chemical factors on the quality of dried blood samples (DBS) during preparation based on 4 neonatal screening assays.

**Methods:** DBS were prepared under 10 different physical and chemical conditions, 7 different concentration gradients of formaldehyde and control conditions. The levels of phenylalanine (Phe), glucose-6-phosphate dehydrogenase (G6PD), thyroid stimulating hormone (TSH) and 17 $\alpha$ -hydroxyprogesterone (17 $\alpha$ -OHP) of different DBS groups were determined by time-resolved fluorescence immunoassay and fluorescence assay. Statistical analysis was performed by SPSS 19.0 software.

**Results:** Compared with the control group, the results of Phe were influenced only when DBS were dried under the formaldehyde condition (4.62~6.95 ppm for 18 h). G6PD levels were significantly decreased when DBS were prepared under all the conditions except fast drying and 2–8 °C overnight. TSH and 17 $\alpha$ -OHP levels were decreased remarkably when DBS were exposed to humidity, UV and formaldehyde (TSH were 1.12~1.27 ppm for 4 h and 0.38~0.45 ppm for 18 h, 17 $\alpha$ -OHP were 4.37~4.62 ppm for 4 h and 0.38~0.45 ppm for 18 h). However, the results of Phe, G6PD, TSH and 17 $\alpha$ -OHP were showed no statistical differences when DBS were dried under fast drying and 2–8 °C overnight in comparison with control.

**Conclusions:** Our results indicated that the accuracy of neonatal screening was strongly affected by the physical and chemical factors during DBS preparation. Especially, exposures to formaldehyde, ethanol, glacial acetic acid, UV, heat, humidity and decoration pollution during preparation significantly influence DBS quality. Furthermore, fast drying and 2–8 °C overnight could be alternative methods for DBS preparation.

*P22. The Effect of Sample Collecting Time on Newborn Screening Thyrotropin*

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**Objective:** To investigate the effect of different sample collecting time on newborn babies. Collecting evidences for 'whether sample collecting time could exceed 72 h'.

**Method:** Gathering the dry blood samples from January to December 2015, detected thyrotropin in dry blood filters.

**Results:** For 2 to 4 day old babies, the concentration of thyrotropin is higher. After 6 days, the concentration of thyrotropin is stable. When using 10 uIU/mL as the cut-off value, there were no difference of screening positive rates and morbidity among the different sample collecting groups.

**Conclusion:** the sample collecting time can affect the thyrotropin. If using an appropriate cut-off value, we could collect the newborn screening samples ahead of 72 h.

*P23. Development and Application of Newborn Screening Information*

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**Objective:** Study the information management of newborn screening. And realize the technical services and information management of newborn screening in Zhejiang province.

**Methods:** Develop a newborn screening information management system based on B/S architecture. It can provide screening technical service and information management for all levels of management centers and production institutions for the newborn screening throughout Zhejiang province

**Results:**

1. Data centralized storage to improve the security of data and fully realize the information sharing.
2. Enables the tracking of logistics throughout the progress of the DBS delivery.
3. Barcode management. The barcode is used as the whole system identification code to establish the screening file.
4. Realize informatization of newborn screening laboratory management.
5. Strengthen long-term follow-up and treatment for positive children.
6. Provide self-service query with website, SMS, WeChat, Alipay.
7. Feature-rich of query statistics to support policy development.

**Conclusion:** The application of newborn screening Information Management System can greatly improve the working efficiency and promote process standardization and scientific administration. It reflects the rapid, accurate, orderly and comprehensive service aim of the newborn screening service.

*P24. The Coverage of Newborn Screening Programme and Quality Control of Dried Blood Spot Test in Mongolia*

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**Introduction:** In the 2000s, with limited funding support from the IAEA, there has been screening programme development around detection of congenital hypothyroidism (CH) in Asia pacific region and one of them was Mongolia—please clarify what you want to say with this sentence. We started

our newborn screening (NBS) programme in 2012 and have screened 13,238 newborns for 5 years in Ulaanbaatar (UB) area.

**Purpose:** To determine the screening coverage of NBS and evaluate dried blood spot samples quality in Ulaanbaatar area.

**Materials and Methods:** We compared the total screening coverage and analysed the quality of dried blood spot samples collected in 2012–2015 and 2016–2017. There were 13,283 newborns in 4 maternal houses in UB with approved parental consent and they were screened for CH and CAH.

**Results:** In 2012–2015, there were 7283 newborns screened for CH and CAH from 80,000 live births and the total screening coverage was 9.1%. In 2016–2017, around 6000 newborns from 40,000 live births were screened for 2 diseases with total screening coverage of 15% showing the increase of number of newborns involved in the screening. CH was detected in 3 cases among 7328 newborns in 2012–2015. CH and subclinical hypothyroidism were detected in 1 with CH and 2 newborns with subclinical hypothyroidism during 2016–2017. The total incidence of CH and subclinical hypothyroidism was 1:2500. In 2016–2017, 2 cases with CAH were detected among 7328 newborns and the incidence was 1:3500. In 2016–2017 2 cases with CAH were detected among 6000 newborns; the incidence was 1:3000. This is the almost same as the result of the previous study in 2012–2015. The following results were analysed and 2421 DBS samples were randomly selected from the 2012–2015 study.

**Conclusions:** At present, the neonatal screening coverage is above 15% in UB city with the same incidence of CH and CAH during these years. Therefore, the newborn screening should be expanded up to national level with the establishment of an NBS programme with quality assurance in order to prevent serious and irreversible consequences, such as severe mental retardation and disability caused by untreated congenital disorders.

#### *P25. Timeliness of Newborn Screening Activities in the Philippines*

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**Background:** Newborn screening is a public health program which aims to detect certain metabolic disorders. The effectiveness of the newborn screening system depends on proper, timely collection of samples and timely diagnosis and appropriate management of cases.

**Objective:** The study aimed to evaluate the newborn screening timeliness, to identify areas in the NBS system that can be shortened to improve timeliness and to identify issues that impact timeliness and how these issues can be addressed by stakeholders.

**Methods:** For 5 years, 5,974,990 newborn infants were screened in the Philippines. Timeliness of newborn screening activities including screening age, transit time, time when NBS results are reported out and turnaround time for immediate notification of medical providers, confirmation of the diagnosis and treatment of cases were reviewed. A five-year data set from the Neometrics database of all centers was obtained retrospectively.

**Results:** The five-year data showed that the percentage of babies screened by Day 2 ranged from 65% to 76%. The percentage of specimens collected beyond 7 days of age decreased within 5 years. The percentage of samples received within 2 days was only 26.6%. However, 95% and 99% were received within 7 and 14 days, respectively. In 96% of cases, normal NBS results were reported out within 5 days from specimen receipt while 98% of out-of-range results were reported out by day 3. Age of confirmation and treatment age for the 6 disorders varied.

**Conclusion:** It is important to routinely monitor the timeliness of NBS activities because this can potentially improve the NBS program performance. The effectiveness of the NBS system can only be achieved if all stakeholders, namely the families, NBS coordinators, health care professionals, newborn screening center staffs and couriers work collectively as a team.



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